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Patent Abstracts of Japan

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PUBLICATION NUMBER : 10298151
PUBLICATION DATE : 10-11-98

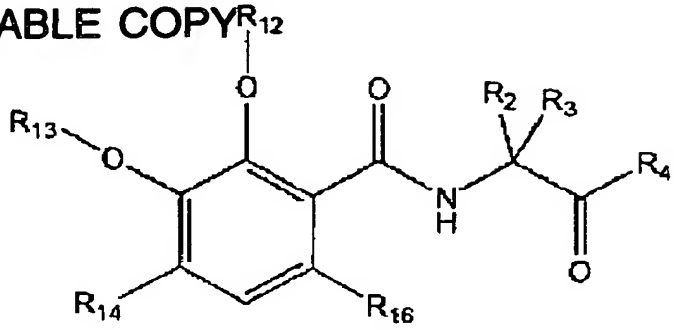
APPLICATION DATE : 30-04-97
APPLICATION NUMBER : 09126462

APPLICANT : JAPAN ENERGY CORP;

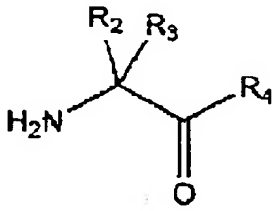
INVENTOR : SAEKI HISAFUMI;

INT.CL. : C07C235/36 A61K 31/27 A61K 31/27 A61K 31/27 A61K 31/27

TITLE : HEPATITIS C VIRUS PROTEASE INHIBITOR



I



II

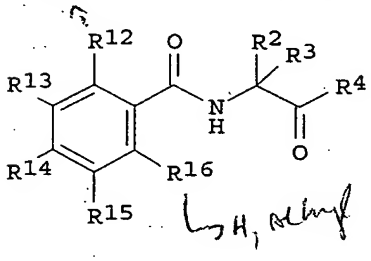
ABSTRACT : PROBLEM TO BE SOLVED: To obtain a new compound which is a specific water-soluble compound, having a relatively low molecular weight and useful as the subject pharmaceutical composition capable of inhibiting enzyme activities of a serine protease derived from hepatitis C viruses.

SOLUTION: This N-substituted benzoylamino acid derivative is represented by formula I (R_2 and R_3 are each H, a 1-6C acyclic hydrogarbon group, etc.; R_4 is H, hydroxyl, etc.; R_{12} and R_{13} are each H or a 1-6C acyclic hydrocarbon group; R_{14} and R_{16} are each H, hydroxyl, etc.) and having dihydroxybenzoyl group or a substituted dihyroxybenzoyl group as a substituent group on the amino group, e.g. N-(2,3-dihydroxybenzoyl)-O-(3-tert-butyloxy-2-(2,3-dihydroxybenzoylamino)propanoyl)serine. The compound represented by formula I is obtained by introducing the substituted benzoyl group into the amino group in an α-amino acid derivative represented by formula II as an intermediate raw material. The compound represented by formula II which is an intermediate raw material is prepared by adding R_4 to the carboxyl terminal of the α-amino acid according to a method used in the peptide synthesis, etc.

L8 ANSWER 7 OF 17 MARPAT COPYRIGHT 2003 ACS on STN
AN 130:33002 MARPAT
TI N-Benzoyl-amino acids, hepatitis C virus protease inhibitors containing them, and therapeutics for hepatitis C
IN Yokota, Tadashi; Ooba, Yoichi; Makita, Atsushi; Saeki, Hisashi
PA Japan Energy K. K., Japan
SO Jpn. Kokai Tokkyo Koho, 25 pp.
CODEN: JKXXAF
DT Patent
LA Japanese
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI JP 10298151	A2	19981110	JP 1997-126462	19970430
PRAI JP 1997-126462		19970430		

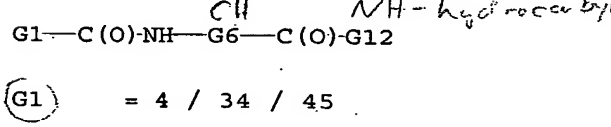
GI



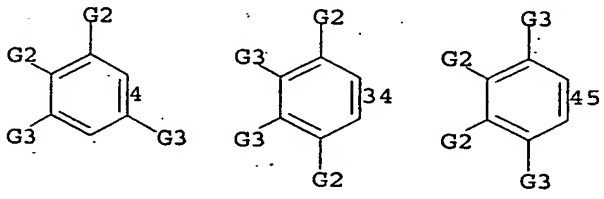
III

AB HCV protease inhibitors contain N-(2,3-dihydroxybenzoyl)serine 3-[2-[(2,3-dihydroxybenzoyl)amino]-2-propenoate] (I), N-(2,3-dihydroxybenzoyl)serine bimol. ester 3-[2-[(2,3-dihydroxybenzoyl)amino]-2-propenoate] (II), III [R2, R3 = H, C1-6 acyclic hydrocarbyl which may have S or O replaced for CH2 o which may be substituted with carboxy, carbamoyl, alkoxycarbonyl, alkyliminocarbonyl, OH, SH, alkylamino, amino, guanidino, aryl, heteroaryl, hydroxyaryl; R2 may represents alkylidene together with R3; R4 = H, OH, SH, amino, (un)substituted hydrocarbyl, hydrocarbyloxy, hydrocarbylthio, hydrocarbylimino, nitrilo; R12, R13 = OH, C1-6 acyclic hydrocarbyloxy; R14, R16 = H, OH, SH, C1-6 acyclic hydrocarbyl, hydrocarbyloxy, hydrocarbylthio; R15 = H] (IV), III (R2-4, R12, R14 = any group given for those in IV, resp.; R13 = any group given for R14 in IV; R15 = H, C1-6 acyclic hydrocarbyl; R16 = H), III (R2-4 = same as above; R12, R15 = any group given for R14 in IV; R13, R14 = OH, C1-6 acyclic hydrocarbyloxy) or their pharmaceutically acceptable salts as active ingredients and useful as treatment of hepatitis C. I and II at 10 .mu.M showed 79.5 and 84.2% inhibition against recombinant HCV protease.

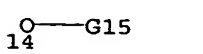
MSTR 1



G1 = 4 / 34 / 45



G2 = OH / 14



G3 = H / OH / SH / Ak<(1-6)> / 18

99-040664/04 B05 NIHA 97.04.30
JAPAN ENERGY CORP *JP 10298151-A
97.04.30 97JP-126462 *(98.11.10)* C07C 235/36, A61K 31/27

Hepatitis C virus protease inhibitors - comprise N-substituted benzoylamino acid derivatives

C99-012550

Hepatitis C virus protease inhibitors comprise N-substituted benzoylamino acid derivatives or their salts containing a partial skeleton belonging to a 2-substituted-2-(dihdyroxybenzoyl)aminoethanoyl structure represented by serine N-(2,3-dihydroxybenzoyl)-bimolecular ester-3-[2-[(2,3-dihydroxybenzoyl)amino]-2-propenoate] of formula (II) or serine N-(2,3-dihdyroxybenzoyl)-3-[2-[(2,3-dihydroxybenzoyl)amino]-2-propenoate] of formula (III) containing a 1-(2,3-dihydroxybenzoyl)amino-2-oxo-ethylene moiety.

USE

The derivatives are useful in the treatment of hepatitis C.

ADVANTAGE

The low-molecular-weight derivatives possess hydro- and lipophilicities leading to high bioavailability, and potent hepatitis C virus-derived serine protease inhibitory activity.
(25pp008DwgNo.0/0)

B(10-C4B, 14-N12) .2

JP 10298151-A

L8 ANSWER 7 OF 17 MARPAT COPYRIGHT 2003 ACS on STN
AN 130:33002 MARPAT
TI N-Benzoyl-amino acids, hepatitis C virus protease inhibitors containing them, and therapeutics for hepatitis C
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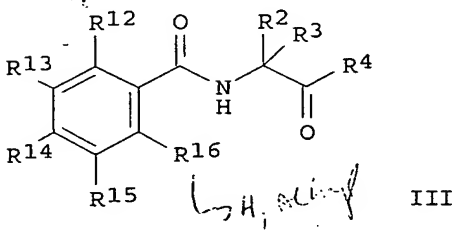
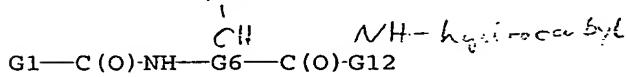
GI



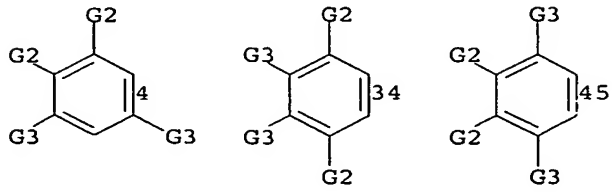
III

AB HCV protease inhibitors contain N-(2,3-dihydroxybenzoyl)serine 3-[2-[(2,3-dihydroxybenzoyl)amino]-2-propenoate] (I), N-(2,3-dihydroxybenzoyl)serine bimol. ester 3-[2-[(2,3-dihydroxybenzoyl)amino]-2-propenoate] (II), III [R2, R3 = H, C1-6 acyclic hydrocarbyl which may have S or O replaced for CH2 o which may be substituted with carboxy, carbamoyl, alkoxycarbonyl, alkyliminocarbonyl, OH, SH, alkylamino, amino, guanidino, aryl, heteroaryl, hydroxyaryl; R2 may represents alkylidene together with R3; R4 = H, OH, SH, amino, (un)substituted hydrocarbyl, hydrocarbyloxy, hydrocarbylthio, hydrocarbylimino, nitrilo; R12, R13 = OH, C1-6 acyclic hydrocarbyloxy; R14, R16 = H, OH, SH, C1-6 acyclic hydrocarbyl, hydrocarbyloxy, hydrocarbylthio; R15 = H] (IV), III (R2-4, R12, R14 = any group given for those in IV, resp.; R13 = any group given for R14 in IV; R15 = H, C1-6 acyclic hydrocarbyl; R16 = H), III (R2-4 = same as above; R12, R15 = any group given for R14 in IV; R13, R14 = OH, C1-6 acyclic hydrocarbyloxy) or their pharmaceutically acceptable salts as active ingredients and useful as treatment of hepatitis C. I and II at 10 .mu.M showed 79.5 and 84.2% inhibition against recombinant HCV protease.

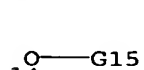
MSTR 1



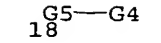
G1 = 4 / 34 / 45



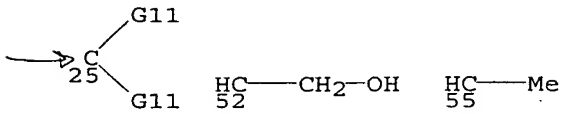
G2 = OH / 14



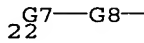
G3 = H / OH / SH / Ak<(1-6)> / 18



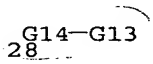
G4 = Ak<(1-6)> / (EX Me)
G5 = O / S
G6 = 25 / Cb<BD (ALL) SE> / (EX 52 / 55)



G7 = Ak<(1-4)>
G8 = O / S
G9 = Ak<(1-4)>
G10 = CO2H / CONH2 / alkoxycarbonyl / alkylaminocarbonyl / OH / SH / alkylamino / dialkylamino / NH2 / NHC(NH)NH2 / aryl (SO OH) / heteroaryl
G11 = H / Ak<(1-6)> (SO G10) / 22



G12 = H / OH / SH / NH2 / hydrocarbyl (SO) / 28 / CN



G13 = hydrocarbyl (SO)
G14 = O / S / NH
G15 = Ak<(1-6)> / (EX Me)
DER: or pharmaceutically acceptable salts
MPL: claim 1

PATENT ABSTRACTS OF JAPAN

(11)Publication number : 10-298151
(43)Date of publication of application : 10.11.1998

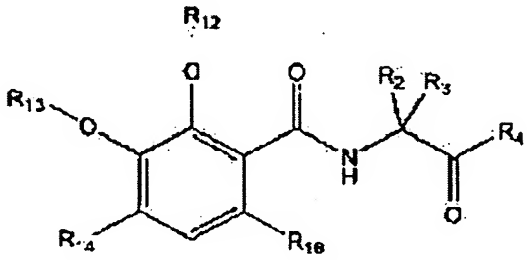
(51)Int.Cl. C07C235/36
A61K 31/27
A61K 31/27
A61K 31/27
A61K 31/27

(21)Application number : 09-126462
(22)Date of filing : 30.04.1997

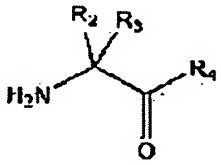
(71)Applicant : JAPAN ENERGY CORP
(72)Inventor : YOKOTA TADASHI
OBA YOICHI
MAKITA ATSUSHI
SAEKI HISAFUMI

(54) HEPATITIS C VIRUS PROTEASE INHIBITOR

(57)Abstract:
PROBLEM TO BE SOLVED: To obtain a new compound which is a specific water-soluble compound, having a relatively low molecular weight and useful as the subject pharmaceutical composition capable of inhibiting enzyme activities of a serine protease derived from hepatitis C viruses.
SOLUTION: This N-substituted benzoylamino acid derivative is represented by formula I (R2 and R3 are each H, a 1-6C acyclic hydrogarbon group, etc.; R4 is H, hydroxyl, etc.; R12 and R13 are each H or a 1-6C acyclic hydrocarbon group; R14 and R16 are each H, hydroxyl, etc.) and having dihydroxybenzoyl group or a substituted dihyroxybenzoyl group as a substituent group on the amino group, e.g. N-(2,3-dihydroxybenzoyl)-O-(3-tert-butyloxy-2-(2,3- dihydroxybenzoylamino)propanoyl)serine. The compound represented by formula I is obtained by introducing the substituted benzoyl group into the amino group in an α-amino acid derivative represented by formula II as an intermediate raw material. The compound represented by formula II which is an intermediate raw material is prepared by adding R4 to the carboxyl terminal of the α-amino acid according to a method used in the peptide synthesis, etc.



I



II

LEGAL STATUS

[Date of request for examination]

[Date of sending the examiner's decision of rejection]

[Kind of final disposal of application other than the examiner's decision of rejection or application converted registration]

[Date of final disposal for application]

[Patent number]

[Date of registration]

[Number of appeal against examiner's decision of

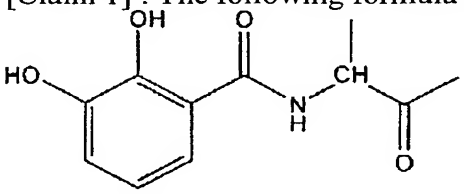
rejection]
[Date of requesting appeal against examiner's decision of rejection]
[Date of extinction of right]

* NOTICES *

1. This document has been translated by computer.So the translation may not reflect the original precisely.
2. **** shows the word which can not be translated.
3.In the drawings, any words are not translated.

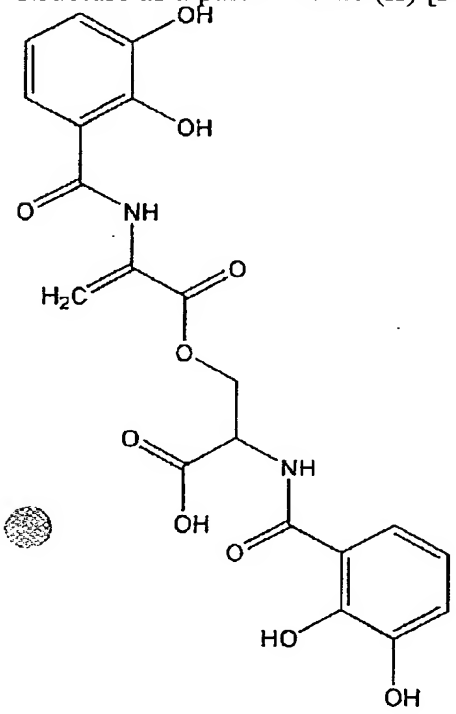
CLAIMS

[Claim(s)]
[Claim 1] : The following formula (I) [Formula 1]



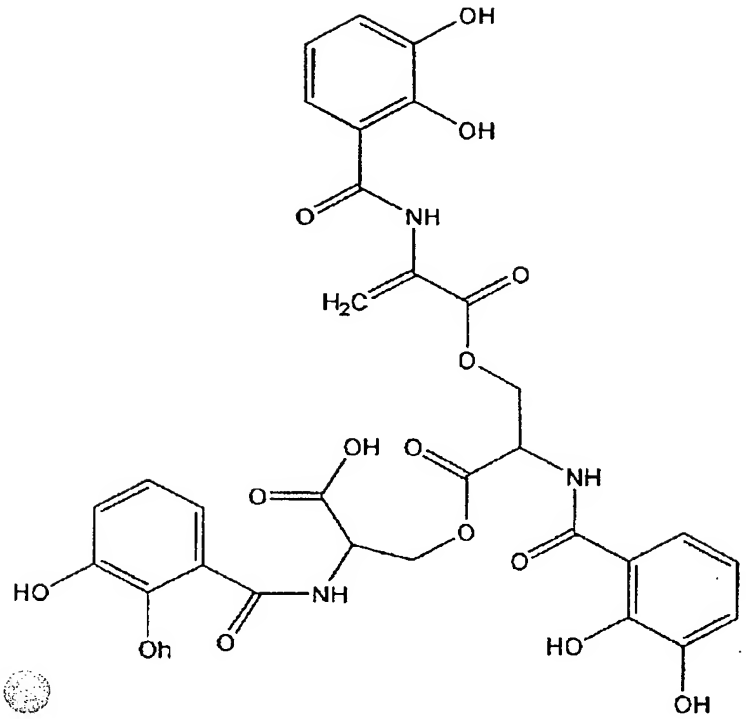
(I)

1-(2, 3-dihydroxybenzoyl) amino -2 - OKISO - : The following formula characterized by including ethylene structure as a partial frame (II) [Formula 2]



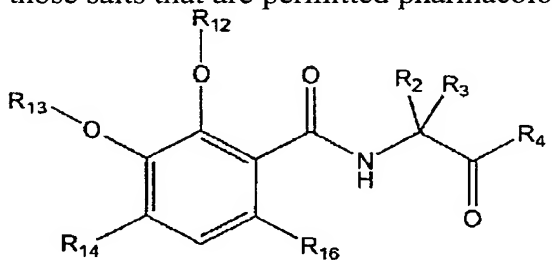
(II)

** Serine, N-(2, 3-dihydroxybenzoyl)-, 3-[2-[(2, 3-dihydroxybenzoyl)amino]-2-propenoate], or formula (III) :. [Formula 3]



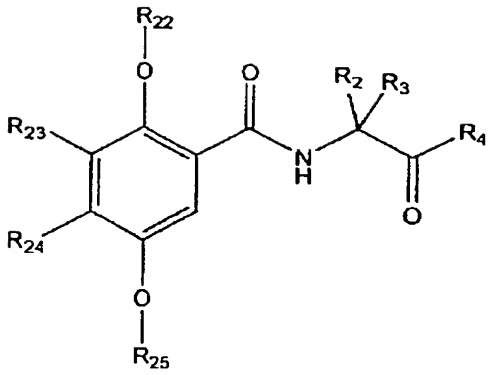
(III)

: ** Serine, N-(2, 3-dihydroxybenzoyl)-, bimol.Ester, 3-[2-[(2, 3-dihydroxybenzoyl)amino]-2-propenoate(s)], those salts that are permitted pharmacologically or the following general formula (IV) [Formula 4]



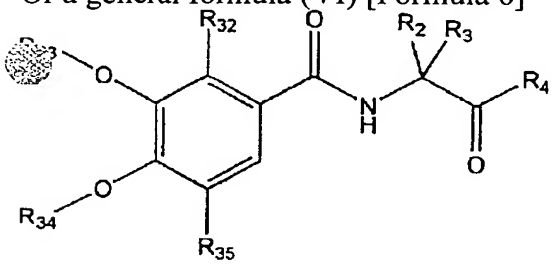
(IV)

(R12 and R13 express a hydrogen atom or the non-cyclic-hydrocarbon machine of carbon numbers 1-6 among a formula, respectively. R14 and R16) Respectively A hydrogen atom, a hydroxyl, a sulfhydryl group, the non-cyclic-hydrocarbon machine of carbon numbers 1-6, The oxy-basis replaced with the non-cyclic-hydrocarbon machine of carbon numbers 1-6 or the thio machine replaced with the non-cyclic-hydrocarbon machine of carbon numbers 1-6 is expressed. R2 and R3 A hydrogen atom, the non-cyclic-hydrocarbon machine of carbon numbers 1-6, the non-cyclic-hydrocarbon machine with which sulfur or oxygen replaced as a thio machine or an oxy-basis in the chain, On the non-cyclic-hydrocarbon machine of the aforementioned carbon numbers 1-6, a carboxyl group, a carbamoyl group, An alkoxy carbonyl group, an alkyl imino carbonyl group, a hydroxyl, The substitution non-cyclic-hydrocarbon machine with which it is replaced any of a sulfhydryl group, the alkylation amino group, the amino group, or a guanidino machine they are, The non-cyclic-hydrocarbon machine replaced with the aromatic-hydrocarbon machine or the complex aromatic ring machine, the non-cyclic-hydrocarbon machine replaced with the hydroxy substitution aromatic-hydrocarbon machine, It expresses any of whether the R2 and R3 concerned exist as a bivalent alkylidene type basis guided from the aforementioned monovalent basis they are. R4 Or a hydrogen atom, Or a hydroxyl, a sulfhydryl group, the amino group, the hydrocarbon group that may be replaced, which monovalent atomic group of the thio machine combined with the oxy-basis combined with the hydrocarbon group which may be replaced, and the hydrocarbon group which may be replaced, the imino group combined with the hydrocarbon group which may be replaced, or a nitrilo group is expressed General formula (V) :. [Formula 5]



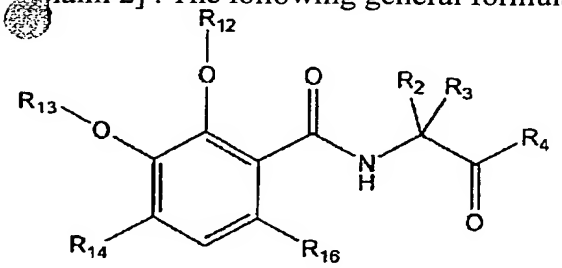
(V)

(R22 and R25 express a hydrogen atom or the non-cyclic-hydrocarbon machine of carbon numbers 1-6 among a formula, respectively. R23 and R24) Respectively A hydrogen atom, a hydroxyl, a sulfhydryl group, the non-cyclic-hydrocarbon machine of carbon numbers 1-6, the oxy-basis replaced with the non-cyclic-hydrocarbon machine of carbon numbers 1-6, or the thio machine replaced with the non-cyclic-hydrocarbon machine of carbon numbers 1-6 -- expressing -- the basis of the R2, R3, and homonymy of the general formula (IV) of the above [R2 and R3] respectively -- expressing -- general formula (IV) of the above [R4] R4 It is homonymy. : Or a general formula (VI) [Formula 6]



(VI)

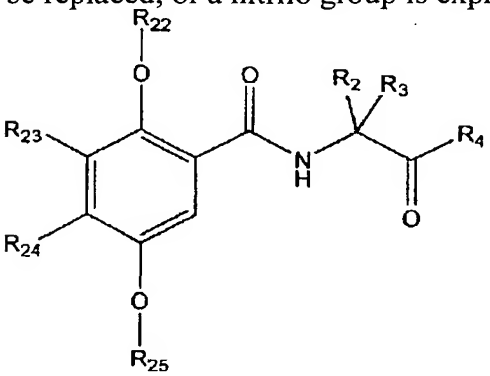
(R33 and R34 express a hydrogen atom or the non-cyclic-hydrocarbon machine of carbon numbers 1-6 among a formula, respectively. R32 and R35) Respectively A hydrogen atom, a hydroxyl, a sulfhydryl group, the non-cyclic-hydrocarbon machine of carbon numbers 1-6, the oxy-basis replaced with the non-cyclic-hydrocarbon machine of carbon numbers 1-6, or the thio machine replaced with the non-cyclic-hydrocarbon machine of carbon numbers 1-6 -- expressing -- the basis of the R2, R3, and homonymy of the general formula (IV) of the above [R2 and R3] respectively -- expressing -- general formula (IV) of the above [R4] R4 It is homonymy. The hepatitis-C-virus protease inhibitor which makes an active principle any of N-substitution benzoylamino acid derivative which has as a substituent the dihydroxybenzoyl machine or substitution dihydroxybenzoyl machine shown depending on any they are on the amino group, or its salt permitted pharmacologically. [Claim 2] : The following general formula (IV) [Formula 7]



(IV)

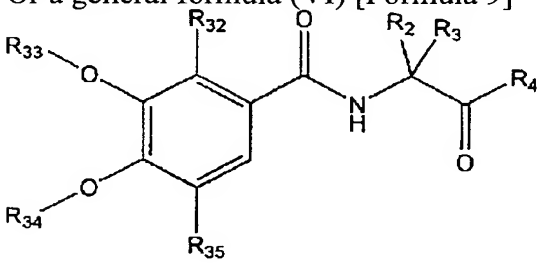
(R12 and R13 express a hydrogen atom or the non-cyclic-hydrocarbon machine of carbon numbers 1-6 among a formula, respectively. R14 and R16) Respectively A hydrogen atom, a hydroxyl, a sulfhydryl group, the non-cyclic-hydrocarbon machine of carbon numbers 1-6, The oxy-basis replaced with the non-cyclic-hydrocarbon machine of carbon numbers 1-6 or the thio machine replaced with the non-cyclic-hydrocarbon machine of carbon numbers 1-6 is expressed. R2 and R3 A hydrogen atom, the non-cyclic-hydrocarbon machine of carbon numbers 1-6, the non-cyclic-hydrocarbon machine with which sulfur or oxygen replaced as a thio machine or an oxy-basis in the chain, On the non-cyclic-hydrocarbon machine of the aforementioned carbon numbers 1-6, a carboxyl group, a carbamoyl group, An alkoxy carbonyl group, an alkyl imino carbonyl group, a hydroxyl, The substitution non-cyclic-hydrocarbon machine with which it is replaced any of a sulfhydryl group, the alkylation amino group, the amino group, or a guanidino machine they are, The non-cyclic-hydrocarbon machine replaced with the aromatic-hydrocarbon machine or the complex aromatic ring machine, the non-

cyclic-hydrocarbon machine replaced with the hydroxy substitution aromatic-hydrocarbon machine, It expresses any of whether the R2 and R3 concerned exist as a bivalent alkylidene type basis guided from the aforementioned monovalent basis they are. R4 Or a hydrogen atom, Or a hydroxyl, a sulfhydryl group, the amino group, the hydrocarbon group that may be replaced, which monovalent atomic group of the thio machine combined with the oxy-basis combined with the hydrocarbon group which may be replaced, and the hydrocarbon group which may be replaced, the imino group combined with the hydrocarbon group which may be replaced, or a nitrilo group is expressed General formula (V) :. [Formula 8]



(V)

(R22 and R25 express a hydrogen atom or the non-cyclic-hydrocarbon machine of carbon numbers 1-6 among formula, respectively. R23 and R24) Respectively A hydrogen atom, a hydroxyl, a sulfhydryl group, the non-cyclic-hydrocarbon machine of carbon numbers 1-6, the oxy-basis replaced with the non-cyclic-hydrocarbon machine of carbon numbers 1-6, or the thio machine replaced with the non-cyclic-hydrocarbon machine of carbon numbers 1-6 -- expressing -- the basis of the R2, R3, and homonymy of the general formula (IV) of the above [R2 and R3] respectively -- expressing -- general formula (IV) of the above [R4] R4 It is homonymy. : Or a general formula (VI) [Formula 9]



(VI)

(R33 and R34 express a hydrogen atom or the non-cyclic-hydrocarbon machine of carbon numbers 1-6 among a formula, respectively. R32 and R35) Respectively A hydrogen atom, a hydroxyl, a sulfhydryl group, the non-cyclic-hydrocarbon machine of carbon numbers 1-6, the oxy-basis replaced with the non-cyclic-hydrocarbon machine of carbon numbers 1-6, or the thio machine replaced with the non-cyclic-hydrocarbon machine of carbon numbers 1-6 -- expressing -- the basis of the R2, R3, and homonymy of the general formula (IV) of the above [R2 and R3] respectively -- expressing -- general formula (IV) of the above [R4] R4 It is homonymy. N-substitution benzoylamino acid derivative characterized by having as a substituent the dihydroxybenzoyl machine or substitution dihydroxybenzoyl machine shown depending on any they are on the amino group, or its salt permitted pharmacologically.

[Claim 3] As an active principle which shows hepatitis C virus protease inhibitory action Serine of the aforementioned formula (II), N-(2, 3-dihydroxybenzoyl)-, and 3- [2-[(2, 3-dihydroxybenzoyl)amino]-2-propenoate] or the aforementioned formula (III) It Serine(s). N-(2, 3-dihydroxybenzoyl)-, bimol.Ester, 3-[2-[(2, 3-dihydroxybenzoyl)amino]-2-propenoate], or those salts that are permitted pharmacologically The hepatitis C therapeutic drug characterized by containing.

[Claim 4] The hepatitis C therapeutic drug characterized by containing N-substitution benzoylamino acid derivative or its salt permitted pharmacologically according to claim 2 as an active principle which shows hepatitis C virus protease inhibitory action.

[Translation done.]

* NOTICES *

1.This document has been translated by computer. So the translation may not reflect the original precisely.
2.**** shows the word which can not be translated.
3.In the drawings, any words are not translated.

DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[The technical field to which invention belongs] this invention relates to the enzyme activity inhibitor of the serine protease of the hepatitis-C-virus origin. Moreover, this invention relates to the physic constituent which makes an active principle the matter which checks the enzyme activity of a hepatitis-C-virus origin serine protease described above, i.e., a hepatitis C therapeutic drug.
[0002]
[Description of the Prior Art] Various medical institutions and research facilities are searched for the virus which participates in the onset of the un-A un-B type chronic hepatitis, and it is clear that the main virus is hepatitis C virus and the thing by which it is classified. Hepatitis C virus which are the cause viruses of blood communicability non-A non-B hepatitis (it is henceforth called HCV for short.) Cause chronic of hepatitis in high frequency, and it is reported that a hepatic carcinoma is advanced (see M.Sakamoto et al., Cancer Res., 48, 7294-7297, etc. (1988)). This genetic information of HCV is already RNA of + chain of about 10 kb. it is clear that close relationship nature, such as a FURABI virus and a Pestivirus, is shown (Q. -L.Choo et al., Science, 244, and 359-362 (1989) --) or -- R.H.Miller & R.H.Purcell, Proc.Natl.Acad.Sci.USA, 87, 2057-2061 (1990), etc. -- reference If the isolation and the analysis result of a base sequence of the HCV viral genome originating in many non-A-non-B-hepatitis patients by various engines are compared, it will have become clear that it can classify in some groups which show local localization. For example, it is the U.S. previously. Chiron The viral genome of HCV originating in the U.S. patient whom the shrine released (see WO 89 / the 046699 grades), By the viral genome of HCV originating in a Japanese patient Differences among some Existing in a base sequence and a corresponding amino acid sequence is reported (). [N.Kato et al., Mol.Biol.Med., 7, 495-501 (1990) or N.Kato etal.,] [Pro.] It is discriminated from the HCV-US type originating in references (1990), such as Natl.Acad.Sci.USA, 87, and 9524-9528, and the U.S. patient, and the HCV-J type originating in a Japanese patient.
[0003] From the analysis of a HCV viral genome, it is reported that the process of the polypropylene theine produced from this genome is carried out by the protease by which the cord is carried out to the signal peptidase and HCV viral genome of host cell nature, and it serves as protein of a gene product. It is reported that especially the RNA polymerase by which a cord is carried out to the non-structural gene field of this HCV viral genome predicted to be required for composition within the host cell of a HCV virus, an RNA helicase, a protease, etc. serve as each enzyme protein in response to cutting by the protease of the HCV virus origin (see Shimotoono et al., a protein nucleic-acid enzyme, 36, 1679-1691, etc. (1991)). In addition, as a translation product of a HCV viral genome, two kinds of proteases exist in a non-structural gene field, and one is METARO Protea. - It is a ZE type. Cpro-1 It is called and other one is a serine protease type. Cpro-2 It is called. latter serine protease type Cpro-2 the process of enzyme protein described above -- involving -- the former METARO Protea-ZE type Cpro-1 participating in cutting between two p Cpro-20 regions is reported Existing non-structural gene field (M.Hijikataet al., J.Virol., 67, and 4665-4675 (1993) --) It exists in p70 region and its amino terminus. or -- M.R.Eckart et al., Biochem.Res.Commun., 192, 399-406 (1993), etc. -- reference In addition, p70 The above included in protein Cpro-2 The amino acid sequence equivalent to a portion of the homology in each interval between roots of HCV is very high, and is reported for the amino acid sequence to cut to be the same (see A.Takamizawa et al., J.Virol., 65, 1105-1113, etc. (1991)).
[0004] in addition, protease of the HCV origin Cpro-2 An amino acid sequence characteristic of the chymotrypsin Mr. serine protease produced from NS3 field of the viral genome of the department of a FURABI virus (: from the amino terminus of a virus polypropylene theine to [ ] the 1075 - 1185th watch Val1074 - Thr1186), In a concrete target, it is more. It is inherent in the substructure of an enzyme activity point as an indispensable portion in the array of His1083-X22-Val1104-X-X-Asp1107-X55-Gly1163-X-Ser1165-Gly1166-

X-Pro1168-X9-Gly1178. In addition, His1083, Asp1107, and Ser1165 which exist in this amino acid sequence are considered to form the substructure of enzyme activity point;His-Asp-Ser of a serine protease. Moreover, protease of the HCV origin Cpro-2 Cys -- Ser And Cys -- Ala Between is cut alternatively and it has the singularity of a cutting amino acid sequence.

[0005] this serine protease type Cpro-2 ** -- since hepatitis-C-virus Protea-ZE (it is henceforth called a HCV protease for short.) called is required for the enzyme activity manifestation of enzyme protein, such as RNA polymerase indispensable to the duplicate proliferation of HCV in a host cell, and an RNA helicase, -- this -- Cpro-2 Virion duplicate proliferation of HCV can be suppressed by checking protease activity. That is, in connection with suppressing virion duplicate proliferation of HCV, it is predicted that it is possible to prevent chronic of hepatitis C. It is this serine protease type at the purpose which prevents chronic of this hepatitis C and is used for medication. Cpro-2 It looks forward to development of the matter which checks protease activity, i.e., a hepatitis-C-virus Protea-ZE inhibitor.

[0006] Since localization especially of the HCV is carried out to liver and virion duplicate proliferation is carried out within a host cell, on the occasion of development of a hepatitis-C-virus Protea-ZE inhibitor, it accumulates in a liver organization out of blood, and it is low molecular weight in comparison, and looks forward to the inhibitor whose shift into a host cell takes place easily and which has water solubility. That is, it is a compound also having the lipophilicity benefited to promote the localization to the liver which is the affected part it to to be not only the aqueous nature child of low molecular weight in comparison, but, and is a HCV protease simultaneously. Cpro-2 It receives and a proposal of the matter which checks the protease activity specifically is desired.

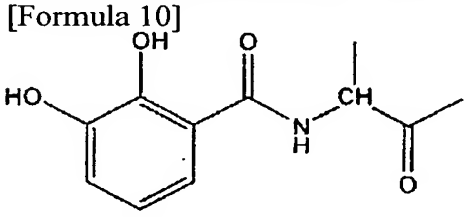
[0007]

[Problem(s) to be Solved by the Invention] this invention solves the aforementioned technical problem and the purpose of this invention is to offer the HCV protease inhibitor which makes the compound concerned an active principle as a new use of the new method of molecular weight which checks the enzyme activity of a hepatitis-C-virus origin serine protease (HCV protease) using a low and a specific water-soluble compound comparatively, i.e., a specific compound. In addition, it is in offering, the physic constituent, i.e., the hepatitis C therapeutic drug, of the hepatitis C treatment use which makes an active principle the compound which has this HCV protease prevention ability.

[0008]

[Means for Solving the Problem] When this invention persons added examination of whether to have HCV protease prevention ability along with the aforementioned purpose to compound a large number which various microorganisms produce, they found out that the HCV protease prevention ability excellent in two or more sorts of microorganism origin compounds was shown. The further examination was added also by making into a selection condition for molecular weight to be in the range which is comparatively small suitable for medicine to two or more sorts of compounds of the microorganism origin in which these HCV(s) protease prevention ability is shown. Consequently, the water-soluble metabolite which contains in the frame N-(dihydroxybenzoyl) serine residue which an Actinomyces produces found out that it was the compound with which are satisfied of these conditions. In addition, in these compounds, HCV protease prevention ability of various similar compounds which has dihydroxybenzoyl structure or its substitution-isomer-ized structure was evaluated and contrasted that an indispensable substructure should be specified for demonstrating HCV protease prevention ability. As a result of this contrast, the compound which has 2, 3-dihydroxybenzoyl structure, 2, 5-dihydroxybenzoyl structure, 3, 4-dihydroxybenzoyl structure, or the structure similar to these as a substructure finds out that HCV protease prevention ability is shown, and came to complete this invention. That is, the hepatitis C virus protease inhibitor of this invention is following formula (I): [0009].

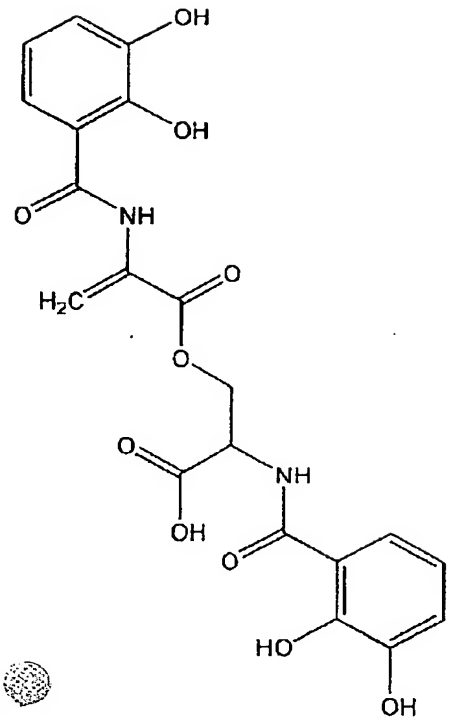
[Formula 10]



(I)

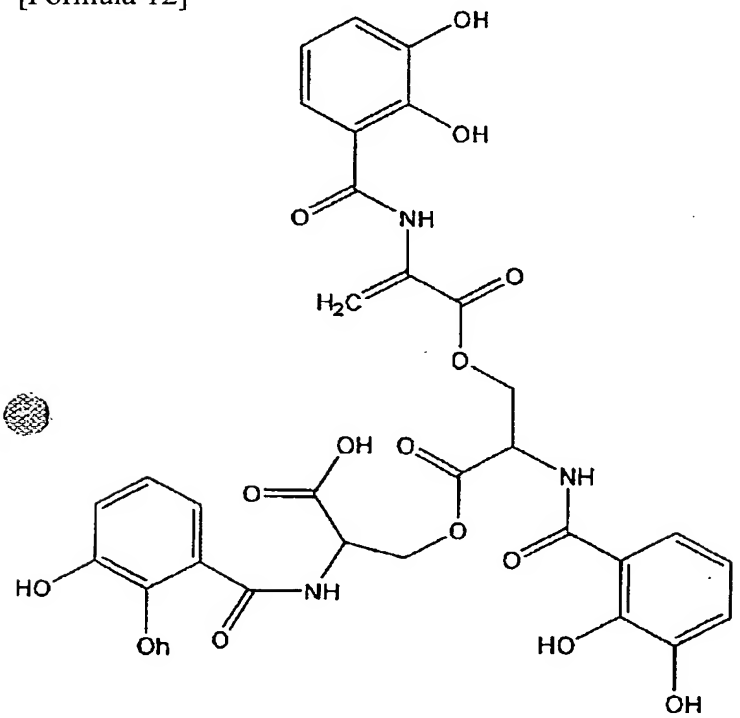
[0010] ** 1-(2, 3-dihydroxybenzoyl) amino -2 - OKISO - The following formula characterized by including ethylene structure as a partial frame (II) : [0011]

[Formula 11]



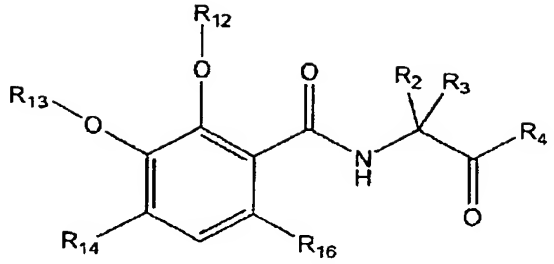
(II)

[0012] ** Serine, N-(2, 3-dihydroxybenzoyl)-, 3-[2-[(2, 3-dihydroxybenzoyl)amino]-2-propenoate], or formula (III) : [0013]
[Formula 12]



(III)

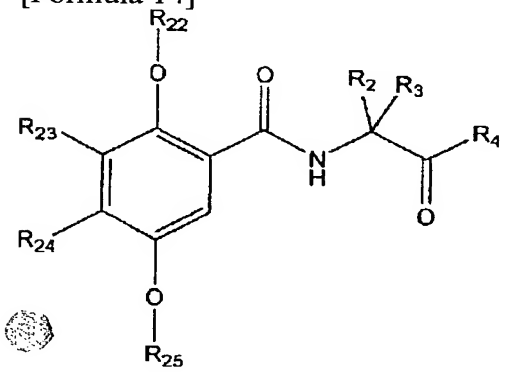
[0014] ** Serine, N-(2, 3-dihydroxybenzoyl)-, bimol.Ester, 3-[2-[(2, 3-dihydroxybenzoyl)amino]-2-propenoate (s)], those salts that are permitted pharmacologically or the following general formula (IV) : [0015]
[Formula 13]



(IV)

[0016] (R12 and R13 express a hydrogen atom or the non-cyclic-hydrocarbon machine of carbon numbers 1-6 among a formula, respectively. R14 and R16) Respectively A hydrogen atom, a hydroxyl, a sulfhydryl group, the non-cyclic-hydrocarbon machine of carbon numbers 1-6, The oxy-basis replaced with the non-cyclic-hydrocarbon machine of carbon numbers 1-6 or the thio machine replaced with the non-cyclic-hydrocarbon machine of carbon numbers 1-6 is expressed. R2 and R3 A hydrogen atom, the non-cyclic-hydrocarbon machine of carbon numbers 1-6, the non-cyclic-hydrocarbon machine with which sulfur or oxygen replaced as a thio machine or an oxy-basis in the chain, On the non-cyclic-hydrocarbon machine of the aforementioned carbon numbers 1-6, a carboxyl group, a carbamoyl group, An alkoxy carbonyl group, an alkyl imino carbonyl group, a hydroxyl, The substitution non-cyclic-hydrocarbon machine with which it is replaced any of a sulfhydryl group, the alkylation amino group, the amino group, or a guanidino machine they are, The non-cyclic-hydrocarbon machine replaced with the aromatic-hydrocarbon machine or the complex aromatic ring machine, the non-cyclic-hydrocarbon machine replaced with the hydroxy substitution aromatic-hydrocarbon machine, It expresses any of whether the R2 and R3 concerned exist as a bivalent alkylidene type basis guided from the aforementioned monovalent basis they are. R4 Or a hydrogen atom, Or a hydroxyl, a sulfhydryl group, the amino group, the hydrocarbon group that may be replaced, which monovalent atomic group of the thio machine combined with the oxy-basis combined with the hydrocarbon group which may be replaced, and the hydrocarbon group which may be replaced, the imino group combined with the hydrocarbon group which may be replaced, or a nitrilo group is expressed General formula (V) : [0017]

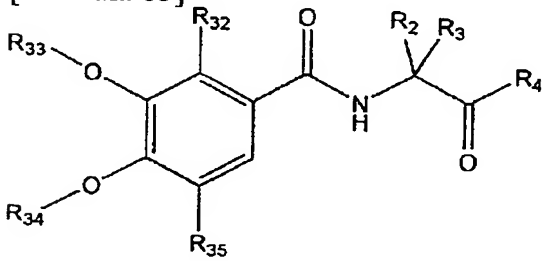
[Formula 14]



(V)

[0018] (R22 and R25 express a hydrogen atom or the non-cyclic-hydrocarbon machine of carbon numbers 1-6 among a formula, respectively. R23 and R24) Respectively A hydrogen atom, a hydroxyl, a sulfhydryl group, the non-cyclic-hydrocarbon machine of carbon numbers 1-6, the oxy-basis replaced with the non-cyclic-hydrocarbon machine of carbon numbers 1-6, or the thio machine replaced with the non-cyclic-hydrocarbon machine of carbon numbers 1-6 -- expressing -- the basis of the R2, R3, and homonymy of the general formula (IV) of the above [R2 and R3] respectively -- expressing -- general formula (IV) of the above [R4] R4 It is homonymy. Or general formula (VI) : [0019]

[Formula 15]



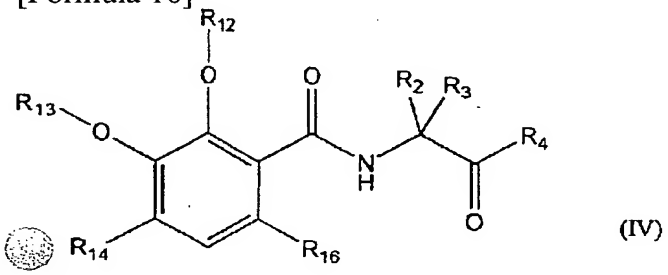
(VI)

[0020] (R33 and R34 express a hydrogen atom or the non-cyclic-hydrocarbon machine of carbon numbers 1-6

among a formula, respectively. R32 and R35) Respectively A hydrogen atom, a hydroxyl, a sulfhydryl group, the non-cyclic-hydrocarbon machine of carbon numbers 1-6, the oxy-basis replaced with the non-cyclic-hydrocarbon machine of carbon numbers 1-6, or the thio machine replaced with the non-cyclic-hydrocarbon machine of carbon numbers 1-6 -- expressing -- the basis of the R2, R3, and homonymy of the general formula (IV) of the above [R2 and R3] respectively -- expressing -- general formula (IV) of the above [R4] R4 It is homonymy. It is the protease inhibitor which makes an active principle any of N-substitution benzoylamino acid derivatives which have as a substituent the dihydroxybenzoyl machine or substitution dihydroxybenzoyl machine shown depending on any they are on the amino group, or those salts that are permitted pharmacologically.

[0021] general formula (IV):[0022 [in addition,] of the following [derivative / N-substitution benzoylamino acid / of this invention / which is used as the active principle in an above-mentioned hepatitis C virus protease inhibitor] --]

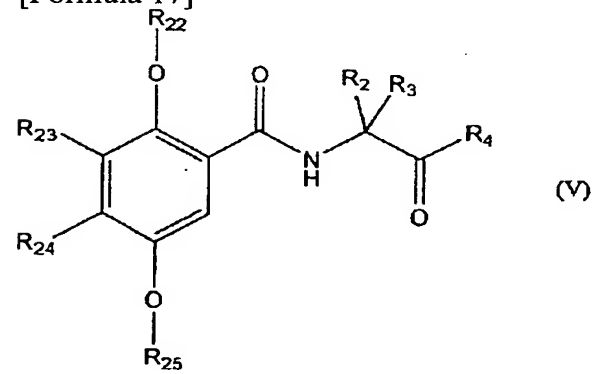
[Formula 16]



(IV)

[0023] (R12 and R13 express a hydrogen atom or the non-cyclic-hydrocarbon machine of carbon numbers 1-6 among a formula, respectively. R14 and R16) Respectively A hydrogen atom, a hydroxyl, a sulfhydryl group, the non-cyclic-hydrocarbon machine of carbon numbers 1-6, The oxy-basis replaced with the non-cyclic-hydrocarbon machine of carbon numbers 1-6 or the thio machine replaced with the non-cyclic-hydrocarbon machine of carbon numbers 1-6 is expressed. R2 and R3 A hydrogen atom, the non-cyclic-hydrocarbon machine of carbon numbers 1-6, the non-cyclic-hydrocarbon machine with which sulfur or oxygen replaced as a thio machine or an oxy-basis in the chain, On the non-cyclic-hydrocarbon machine of the aforementioned carbon numbers 1-6, a carboxyl group, a carbamoyl group, An alkoxy carbonyl group, an alkyl imino carbonyl group, a hydroxyl, The substitution non-cyclic-hydrocarbon machine with which it is replaced any of a sulfhydryl group, the alkylation amino group, the amino group, or a guanidino machine they are, The non-cyclic-hydrocarbon machine replaced with the aromatic-hydrocarbon machine or the complex aromatic ring machine, the non-cyclic-hydrocarbon machine replaced with the hydroxy substitution aromatic-hydrocarbon machine, It expresses any of whether the R2 and R3 concerned exist as a bivalent alkylidene type basis guided from the aforementioned monovalent basis they are. R4 Or a hydrogen atom, Or a hydroxyl, a sulfhydryl group, the amino group, the hydrocarbon group that may be replaced, which monovalent atomic group of the thio machine combined with the oxy-basis combined with the hydrocarbon group which may be replaced, and the hydrocarbon group which may be replaced, the imino group combined with the hydrocarbon group which may be replaced, or a nitrilo group is expressed General formula (V) : [0024]

[Formula 17]

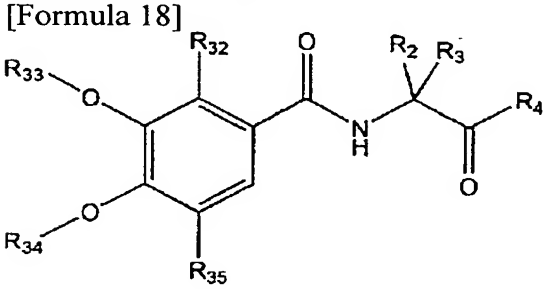


(V)

[0025] (R22 and R25 express a hydrogen atom or the non-cyclic-hydrocarbon machine of carbon numbers 1-6 among a formula, respectively. R23 and R24) Respectively A hydrogen atom, a hydroxyl, a sulfhydryl group, the non-cyclic-hydrocarbon machine of carbon numbers 1-6, the oxy-basis replaced with the non-cyclic-hydrocarbon machine of carbon numbers 1-6, or the thio machine replaced with the non-cyclic-hydrocarbon machine of carbon numbers 1-6 -- expressing -- the basis of the R2, R3, and homonymy of the general formula

(IV) of the above [R2 and R3] respectively -- expressing -- general formula (IV) of the above [R4] R4 It is homonymy. Or general formula (VI) : [0026]

[Formula 18]



(VI)

[0027] (R33 and R34 express a hydrogen atom or the non-cyclic-hydrocarbon machine of carbon numbers 1-6 among a formula, respectively. R32 and R35) Respectively A hydrogen atom, a hydroxyl, a sulfhydryl group, the non-cyclic-hydrocarbon machine of carbon numbers 1-6, the oxy-basis replaced with the non-cyclic-hydrocarbon machine of carbon numbers 1-6, or the thio machine replaced with the non-cyclic-hydrocarbon machine of carbon numbers 1-6 -- expressing -- the basis of the R2, R3, and homonymy of the general formula (IV) of the above [R2 and R3] respectively -- expressing -- general formula (IV) of the above [R4] R4 It is homonymy. It is N-substitution benzoylamino acid derivative which has as a substituent the dihydroxybenzoyl machine or substitution dihydroxybenzoyl machine shown depending on any they are on the amino group.

[0028] Moreover, one mode of the hepatitis C therapeutic drug of this invention is a physic constituent containing N-substitution benzoylamino acid derivative shown by the aforementioned general formula (IV), (V), or (VI), or its salt permitted pharmacologically as an active principle which shows hepatitis-C-virus protease inhibitory action. Furthermore, other modes of the hepatitis C therapeutic drug of this invention As an active principle which shows hepatitis-C-virus protease inhibitory action Serine of the aforementioned formula (II), N-(2, 3-dihydroxybenzoyl)-, and 3- [2-[(2, 3-dihydroxybenzoyl)amino]-2-propenoate] or formula (III) It Serine(s). N-(2, 3-dihydroxybenzoyl)-, bimol.Ester, 3-[2-[(2, 3-dihydroxybenzoyl)amino]-2-propenoate], or those salts that are permitted pharmacologically It is the physic constituent to contain.

[0029]

[Embodiments of the Invention] N-substitution benzoylamino acid derivative compounds which is used for the hepatitis C virus protease inhibitor of this invention and which are shown by the general formula (IV), (V), or (VI) An aforementioned formula (II) or an aforementioned formula (III) The characteristic substructure which is inherent in two sorts of compounds shown, namely, from compound a large number which have N-substitution benzoylamino acid frame which has a dihydroxybenzoyl machine as a substituent on the amino group, and a similar substructure A suitable common substructure to demonstrate the outstanding HCV protease prevention ability is extracted and specified, and it invents, and is found out.

[0030] the compound specifically expressed with an above-mentioned general formula (IV) -- a formula (II) or formula (III) 1-(2, 3-dihydroxybenzoyl) amino-2- of a formula (I) which is the characteristic substructure which is inherent in two sorts of compounds shown the relative holding the structure from which oxo ethylene structure or its hydroxyl was protected with non-cyclic-hydrocarbon machines, such as an alkyl group, -- it is a compound group Namely, the hydroxyl to which R12-O- and R13-O- exist in the 2nd place on the benzoyl of the compound concerned, and the 3rd place, Or the non-cyclic-hydrocarbon machine which was protected with the non-cyclic-hydrocarbon machine and which is a hydroxyl and is chosen in these R12 and R13 It is chosen from the range of carbon numbers 1-6. The alkyl group of a saturated-hydrocarbon machine for example, a methyl group, an ethyl group, a propyl group, an isopropyl machine, and a butyl -- A pentyl machine, a hexyl machine or the corresponding ARUKENIRU machine (for example, a vinyl group, a propenyl machine, a butenyl group, a pentenyl machine, a hexenyl machine) of an unsaturation hydrocarbon group, an alkadienyl machine (for example, pig dienyl machine), etc. can be used. In addition, that from which R12-O- and R13-O- do not produce steric hindrance mutually, for example, a hydroxyl, and a methoxy machine are more desirable, at least, when R12-O- chooses a hydroxyl, it is still more desirable, and it is much more desirable in their being both hydroxyls.

[0031] On the other hand, the basis R14 which exists in the 4th place of this benzoyl, and the basis R16 which exists in the 6th place are chosen from the oxy-basis replaced with a hydrogen atom, the hydroxyl, the sulfhydryl group, the non-cyclic-hydrocarbon machine of carbon numbers 1-6, and the non-cyclic-hydrocarbon machine of carbon numbers 1-6, and the thio machine replaced with the non-cyclic-hydrocarbon machine of carbon numbers 1-6. In addition, in a basis R14, a hydrogen atom, a hydroxyl, or a sulfhydryl group is still more desirable on adjoining atomic-group R13-O-, the thing which does not produce steric hindrance mutually, a thing without producing [in / a basis R16 / similarly / a hydrogen atom a hydroxyl, or a sulfhydryl group is

specifically still more desirable, and] steric hindrance, and a concrete target.
[0032] Moreover, the bases R2 and R3 which exist on 1-amino-2-OKISO-ethylene structure A hydrogen atom, the non-cyclic-hydrocarbon machine of carbon numbers 1-6, the non-cyclic-hydrocarbon machine with which sulfur or oxygen replaced as a thio machine or an oxy-basis in the chain, On the non-cyclic-hydrocarbon machine of the aforementioned carbon numbers 1-6, a carboxyl group, a carbamoyl group, An alkoxy carbonyl group, an alkyl imino carbonyl group, a hydroxyl, The substitution non-cyclic-hydrocarbon machine with which it is replaced any of a sulfhydryl group, the alkylation amino group, the amino group, or a guanidino machine they are, [whether it is chosen from the non-cyclic-hydrocarbon machine replaced with the non-cyclic-hydrocarbon machine and hydroxy substitution aromatic-hydrocarbon machine which were replaced with the aromatic-hydrocarbon machine or the complex aromatic ring machine, and] Or bases R2 and R3 are in any of whether to exist as a bivalent alkylidene type basis guided from the aforementioned monovalent basis. That is, 1-amino-2-OKISO-ethylene structure forms natural alpha-amino acid residue, or it and similar artificial alpha-amino acid residue. In addition, it is a hydrogen atom any of bases R2 and R3 they are, or this selection [which / whether it exists as a bivalent alkylidene type basis and] is desirable.
[0033] Specifically as a non-cyclic-hydrocarbon machine of the aforementioned carbon numbers 1-6 the alkyl group (for example, a methyl group, an ethyl group, a propyl group, and an isopropyl machine --) of saturation A butyl, an isobutyl machine, a sec-butyl, t-butyl, a pentyl machine, A hexyl machine, the corresponding alkenyl machine (for example, a vinyl group, a propenyl machine, a butenyl group, a pentenyl machine, a hexenyl machine) of an unsaturation, etc. are more desirable, and the non-cyclic-hydrocarbon machine with which sulfur or oxygen replaced as a thio machine or an oxy-basis in the chain is also more desirable. Moreover, the number of the carboxyl group replaced by the non-[these] cyclic-hydrocarbon machine, a carbamoyl group, an alkoxy carbonyl group, an alkyl imino carbonyl group, a hydroxyl, a sulfhydryl group, the alkylation amino group, the amino group, or guanidino machines has one more desirable thing. As the aromatic-hydrocarbon machine similarly replaced by the non-cyclic-hydrocarbon machine, a complex aromatic ring machine, or a hydroxy substitution aromatic-hydrocarbon machine, it becomes what has the thing of the phenyl group looked at by the phenylalanine, p-hydroxyphenyl machine looked at by the thyrosin, the indolyl machine looked at by the tryptophan, the imidazolyl machine looked at by the histidine, or the structure similar to this desirable [more] the thing of a condensation 2 ring type or a monocycle formula for example, more desirable. Furthermore, these bases R2 and R3 have that still more desirable by which steric hindrance must have been mutually produced with the substitution benzoyl on the adjoining amino group, combination between molecules, for example, hydrogen bond, must have been formed between mutual substituents, or a limit cannot join the orientation of a substitution benzoyl according to these factors.
[0034] Therefore, the glycine classified into hydrophobic amino acid among the natural alpha-amino acid, On a filling [hydrophobic]-like side chain of alanine, valine, leucine, isoleucine, methionine, phenylalanine, and tryptophan thing, and a concrete target The alkyl group of saturation, the corresponding alkenyl machine of an unsaturation, a thia alkyl group, An OKISA alkyl group, a corresponding thia alkenyl machine, an OKISA alkenyl machine, A phenyl substitution alkyl group, a phenyl substitution alkenyl machine, etc.; Are classified to a neutral amino acid. Like the side chain of a serine, a threonine, a cysteine, a thyrosin, an asparagine, and a glutamine On the thing and concrete target which have only the functional group which does not show the work as an acid or a base although it is hydrophilic A hydroxyalkyl machine, a corresponding hydroxy alkenyl machine, a mercapto alkyl group, A mercapto alkenyl machine, p-hydroxyphenyl substitution alkyl group, p-hydroxyphenyl substitution alkenyl machine, Or a carbamoyl alkyl group, a carbamoyl alkenyl machine, an alkoxy carbonyl alkyl group, An alkoxy carbonyl alkenyl machine, an alkyl imino carbonyl alkyl group, an alkyl imino carbonyl alkenyl machine, etc. can be mentioned as an example, although it is still more desirable. In addition, as well as the natural alpha-amino acid, although it is still more desirable when it is the basis which is similar to a hydrogen atom in one side of bases R2 and R3, and is similar to the aforementioned hydrophobic property, the side chain of a neutral amino acid, or it in another side, when the configuration serves as L-object, it is much more desirable.
[0035] Subsequently, R4 is equivalent to an alpha-amino-acid carboxyl terminus. Originally, it is the protease of the HCV origin. Cpro-2 Cys which exists in the polypeptide of the HCV origin -- Ser And Cys -- Ala Between is cut alternatively and they are above Ser and Ala. A fairly long peptide chain exists in a carboxyl terminus. A part for the 1-amino-2-OKISO-ethylene structured division to which R4 is added is this Ser and Ala. As it can be regarded as the thing equivalent to an alpha-amino-acid residue and a substrate peptide sees, you may be the fairly huge atomic group connected by amide combination. Or the formula (II) or formula described above (III) Like the compound shown, you may be the fairly huge atomic group connected by ester combination. Moreover, even if it is the structure connected by thioester combination, you may be the structure connected with the direct chain.

[0036] That is, R4 can be chosen from any of which monovalent atomic group of the thio machine combined with a hydrogen atom or a hydroxyl, a sulfhydryl group, the amino group, the hydrocarbon group that may be replaced, the oxy-basis combined with the hydrocarbon group which may be replaced, and the hydrocarbon group which may be replaced, the imino group combined with the hydrocarbon group which may be replaced, or a nitrilo group. For example, when R4 can express it as bivalent basis-X- and the atomic group which consists of monovalent atomic groups R5, bivalent basis-X- which constitutes -X-R5 can be chosen from either an imino group (-NH-), an oxy-basis (-O-), a thio machine (-S-) or a methylene group (-CH2-). In addition, you may be the amino group replaced by two hydrocarbon groups like the thing to which it changes to an imino group (-NH-), for example, a nitrogen-carbon to carbon bond exists further like the proline which is the annular alpha-amino acid, and the 2 substitution amino group exists in a ring structure like the thing used as a nitrilo group, for example, a pyrrolidino machine, (1-pyrrolidyl machine), a piperidino machine (1-piperidyl machine), etc., or a dialkylamino machine. You may be the non-cyclic-hydrocarbon machine with which it replaces with a methylene group, a carbon-carbon to carbon bond similarly exists further, and a carbon-carbon double bond exists between the 1st place, such as non-cyclic-hydrocarbon machines which have [of the 1st place] branching, such as a thing to which this methylidyne machine exists in ring structures, such as a cyclic-hydrocarbon machine, like the thing used as a methylidyne machine, for example, a cyclopentylic group, a cyclohexyl machine, etc., or an isopropyl machine, and 1-propenyl machine, and the 2nd place Furthermore, R4 is a hydrogen atom, i.e., this portion may serve as a formyl machine.

[0037] The 1-(substitution benzoyl) amino-2-oxo ethylene structure itself which these R4 combines has that desirable from which it is equivalent to the dipeptide fragment of a substrate cleavage site, and the whole molecule size including these R4 turns into molecule size of the same grade as tripeptide. If it puts in another way, the molecule size which can use the dipeptide transport system or tripeptide transport system which is a matter transportation path into a cell in a living body will be desirable. Therefore, as for R4, it is desirable to consider as the range which does not exceed greatly 12 of an arginine which does not have 14 or the cyclic structure of a tryptophan including the grade as natural amino acid that total of the skeleton atomic number which constitutes it is the same, for example, a cyclic structure, and these skeleton atomic numbers. Therefore, R4 has the desirable atomic group which corresponds further when R5 is hydrocarbon groups other than a hydrogen atom etc., the amino group (-NH2) and hydroxyl (-OH) which correspond when a hydrogen atom or R4 can express in the form of -X-R5 and R5 is a hydrogen atom, a sulfhydryl group (-SH) or a methyl group (-CH3), and and which is described below. In that whose R5 is hydrocarbon groups other than a hydrogen atom etc. The non-cyclic-hydrocarbon machine which the carbon number of the maximum chain is seven or less range, and does not exceed 12 including the carbon number of the side chain except for X in which a residual valence exists, Or the basis guided in the hetero atom substitution of a nitrogen atom, an oxygen atom, and a sulfur atom from the hydrocarbon group which has the hydrocarbon group, the further aforementioned non-cyclic-hydrocarbon machine, or ring structure which has the ring structure to which the number of total carbons does not exceed 14 including X including the ring which does not exceed condensation 2 ring is desirable.

[0038] The compound expressed with the compound and general formula (VI) which are expressed with a general formula (V) replaces the substitution benzoyl portion of the compound expressed with the general formula (IV) mentioned above by other substitution benzoyls, respectively, and the structures R2, R3, and R4 of the portion except the substitution benzoyl concerned, i.e., bases, are a thing in a general formula (IV), and the thing of homonymy. Moreover, in each of bases R2, R3, and R4, the more desirable range also becomes the same thing as the range explained in the general formula (IV).

[0039] The substitution benzoyl in the compound expressed with a general formula (V) holds the structure from which 2 and 5-dihydroxybenzoyl structure or its hydroxyl was protected with non-cyclic-hydrocarbon machines, such as an alkyl group. Namely, the hydroxyl to which R22-O- and R25-O- exist in the 2nd place on the benzoyl of the compound concerned, and the 5th place, Or the non-cyclic-hydrocarbon machine which was protected with the non-cyclic-hydrocarbon machine and which is a hydroxyl and is chosen in these R22 and R25 It is chosen from the range of carbon numbers 1-6, and the alkyl group of a saturated-hydrocarbon machine or the corresponding alkenyl machine of an unsaturation hydrocarbon group, an alkadienyl machine, etc. can be used. In addition, as that from which R23 which exists in the 3rd place of R22-O- and a benzoyl does not produce steric hindrance mutually, for example, R22-O-, a hydroxyl and a methoxy machine are more desirable, and when a hydroxyl is chosen, it is still more desirable.

[0040] A hydroxyl and a methoxy machine are more desirable, and as R24 to which basis R25-O- which exists in the 5th place of this substitution benzoyl also exists in the 4th place of a benzoyl on the other hand and the thing which does not produce steric hindrance mutually, for example, R25-O-, when a hydroxyl is chosen, it is still more desirable. 3 which remains The basis R23 which exists in grade, and the basis R24 which exists in the 4th place are chosen from the thio machine replaced with the oxy-basis replaced with the hydrogen atom, the

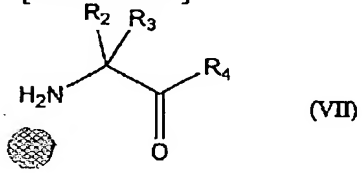
hydroxyl, the non-cyclic-hydrocarbon machine of carbon numbers 1-6, and the non-cyclic-hydrocarbon machine of carbon numbers 1-6, the sulfhydryl group, and the non-cyclic-hydrocarbon machine of carbon numbers 1-6. In addition, also in bases R23 and R24, on the adjoining substituent, the thing which does not produce steric hindrance mutually, and a concrete target, a hydrogen atom, a hydroxyl, a sulfhydryl group, and a methyl group are still more desirable, and even if there are few bases R23 and R24, when it makes into a hydrogen atom any to be, on them, it is much more desirable.

[0041] The substitution benzoyl in the compound expressed with a general formula (VI) holds the structure from which 3 and 4-dihydroxybenzoyl structure or its hydroxyl was protected with non-cyclic-hydrocarbon machines, such as an alkyl group. Namely, the hydroxyl to which R33-O- and R34-O- exist in the 3rd place on the benzoyl of the compound concerned, and the 4th place, Or the non-cyclic-hydrocarbon machine which was protected with the non-cyclic-hydrocarbon machine and which is a hydroxyl and is chosen in these R33 and R34 It is chosen from the range of carbon numbers 1-6, and the alkyl group of a saturated-hydrocarbon machine or the corresponding alkenyl machine of an unsaturation hydrocarbon group, an alkadienyl machine, etc. can be used. In addition, what does not produce steric hindrance mutually, for example, a hydroxyl, and its methoxy machine are more desirable, and when R33-O- and R34-O- choose a hydroxyl for any being at least, they are still more desirable.

[0042] On the other hand, the basis R32 which exists in the 2nd place and the basis R35 which exists in the 5th place in which this substitution benzoyl remains are chosen from the oxy-basis replaced with a hydrogen atom, the hydroxyl, the sulfhydryl group, the non-cyclic-hydrocarbon machine of carbon numbers 1-6, and the non-cyclic-hydrocarbon machine of carbon numbers 1-6, and the thio machine replaced with the non-cyclic-hydrocarbon machine of carbon numbers 1-6. In addition, also in bases R32 and R35, on the adjoining substituent, the thing which does not produce steric hindrance mutually, and a concrete target, a hydrogen atom, a hydroxyl, a sulfhydryl group, and a methyl group are still more desirable, and even if there are few bases R33 and R35, when it makes into a hydrogen atom any to be, on them, it is much more desirable.

[0043] The inside of N-substitution benzoylamino acid derivative compounds shown by the general formula (IV), (V), or (VI), Respectively 2, 3-dihydroxybenzoyl machine, 2, 5-dihydroxybenzoyl machine, What non-cyclic-hydrocarbon machines, such as an alkyl group, replace on the hydroxyl of 3 and 4-dihydroxybenzoyl machine In itself, although enzyme-inhibition activity is not necessarily high, with in-the-living-body metabolism, a non-cyclic-hydrocarbon machine is omitted, it is changed into a hydroxyl, and medicinal action is demonstrated. That is, a hydroxyl is protected and it is equivalent to the compound formed into the PURODO rack. Each N-substitution benzoylamino acid derivative compound shown by the general formula (IV), (V), or (VI) is the following general formula. (VII) : [0044]

[Formula 19]



(VII)

[0045] (-- R2, R3, and R4 are the above and homonymy among a formula It can manufacture by N-benzoylation reaction by introducing the substitution benzoyl corresponding to the amino-group top, respectively by using as a middle raw material the alpha-amino-acid derivative shown by). Moreover, general formula of a middle raw material (VII) The alpha-amino-acid derivative itself shown is easily prepared using the technique used widely in peptide synthesis etc. by adding R4 to the carboxyl terminus of the alpha-amino acid concerned. in addition, in these condensation reactions, by that in which the functional group in which side reaction occurs exists, after protecting the functional group concerned beforehand and reacting the purpose, it comes out not to mention carrying out a deprotection For example, after protecting the amino group of the alpha-amino acid, and forming amide combination, a deprotection can be carried out and the alpha-amino-acid derivative of a middle raw material can be obtained [when R4 is a substitution amino group,].

[0046] In addition, since the substituent as which they are characterized by N-substitution benzoylamino acid derivative compounds prepared by these chemosynthesis originates in a start raw material, the identification is a spectroscopy-means, for example, NMR. It can carry out easily in analyzing these measurement results, referring to a start raw material using a method and the IR method.

[0047] The inside of the compound in which the HCV protease prevention ability used for the hepatitis C therapeutic drug of this invention is shown, Serine of the aforementioned formula (II), N-(2, 3-dihydroxybenzoyl)-, and 3- [2-[(2, 3-dihydroxybenzoyl)amino]-2-propenoate] or formula (III) It Serine(s). Each of N-(2, 3-dihydroxybenzoyl)-, bimol.Ester(s), and 3-[2-[(2, 3-dihydroxybenzoyl)amino]-2-propenoate]

A microorganism, Streptomyces longisporous JCM4261 Although it is the compound of the stock origin, it is the known compound already isolated and reported from other microorganisms. In addition, aforementioned Streptomyces longisporous JCM4261 A stock is strain saved in the Institute of Physical and Chemical Research microorganism preservation-of-line institution, and can receive sale in lots from the institution concerned.
[0048] In addition, the inside of Chemical Abstracts Registry File in which a known compound is mentioned, Serine of a formula (II), N-(2, 3-dihydroxybenzoyl)-, and 3- L-object of [2-[(2, 3-dihydroxybenzoyl)amino]-2-propenoate] As a compound of the registry number 73410-35-2 Serine of a formula (III), N-(2, 3-dihydroxybenzoyl)-, bimol.Ester, and 3- L of [2-[(2, 3-dihydroxybenzoyl)amino]-2-propenoate], and L-object As a compound of the registry number 30567-78-2, it is recorded, respectively.
[0049] These Streptomyces longisporous JCM4261 Two sorts of natural compounds which a stock produces have a common feature in alpha-amino group of alpha-amino acid residue at the point which 2 and 3-dihydroxybenzoyl machine replaces. Although it is also extractable by how (see Biochim.Biophys.Acta (1970), 215 (2), and -402 or the 393 patent [Europe] EP No. 5346 official report) to already have reported reference, it is aforementioned Actinomyces Streptomyces longisporous JCM4261. A stock can be cultivated and can also be isolated from the culture medium according to the procedure which carries out the following.
[0050] Streptomyces longisporous JCM4261 Isolation method 1. cultivation this Actinomyces from stock culture medium The amount of biomass 1 loops which cultivated 4261 stocks of JCM(s) on YM plate (0.4% [of yeast extracts], 1.0% [of malt extracts], and glucose 0.4%, 2.0% of agars, and pH 7.3) is inoculated into 5ml (24phi test tubes) of culture medium of the composition which carries out the following. Shaking culture is carried out for two days, and 30 degrees C of preculture liquid are obtained. 5ml of this preculture liquid is added to 100ml (500ml Sakaguchi flask) of culture medium of the same composition, and it carries out shaking culture for five days 30 degrees C.
[0051]
[Table 1]

培地組成	含有率
可溶性でんぷん	2.0 %
グルコース	2.0 %
NaCl	0.1 %
K_2HPO_4	0.1 %
$MgSO_4 \cdot 7H_2O$	0.1 %
$CaCO_3$	0.05%
Bacto Soytone	1.0 %
	pH 7.2

[0052] 2. Carry out centrifugal separation (6000 rpm, 30 min) of the culture medium of the separation above, add 6N HCl to a supernatant liquid, and adjust to pH 2.0. Equivalent ethyl acetate performs a solvent extraction 3 times. Re-extraction is performed in equivalent saturation sodium-hydrogencarbonate solution from the extracted ethyl-acetate layer. It is HCl to the water layer obtained. It adds, and adjusts to pH 2.0 and equivalent ethyl acetate extracts again. Then, this ethyl-acetate layer is dehydrated by anhydrous sodium sulfate, it hardens by drying, and an acid extraction fraction is obtained.
[0053] An acid extraction fraction is dissolved in a methanol, it is easy to add and silica gel 216 mg is stirred. A methanol is removed by the evaporator and an acid extraction fraction is made to stick to silica gel. You put the silica gel which adsorbed the aforementioned acid extraction fraction on the overhead of the open column which packed silica gel 6.48 g, and make it eluted by the step gradient of a chloroform-ethyl-acetate-methanol, and separation extraction of each fraction is carried out. The two aforementioned sorts of target compounds are contained in the fraction eluted with an ethyl-acetate-methanol (9:1) solution. In the HPLC analysis result of drawing 1 , it is separated from this ethyl-acetate-methanol elution fraction of 9:1 by which fractionation was carried out as a peak of elution time 25.3 min and 29.7 min by the HPLC conditions which carry out the following.
[0054] HPLC analysis condition column: YMC-Pack, ODS-AM, 150 x4.6 mm (YMC Co., Ltd)
Solvent: The smaller compound of a formula (II) of molecular weight is the peak of elution time 25.3 min in a 10% acetonitrile (0.1% TFA)-50% acetonitrile (0.1% TFA) / 40-minute a linear gradient, i.e., drawing 1 , and it is the larger formula of molecular weight. (III) A compound is the peak of elution time 29.7 min. In addition, the compound of a formula (II) is a formula. (III) Although it is equivalent to that by which N-(2, 3-dihydroxybenzoyl) serine 1 molecule of a composition unit was removed from the compound, if HPLC analysis

of the culture supernatant itself is carried out, the peak equivalent to two sorts of these compounds will be found out, for example as shown in drawing 2 . That is, in a isolation course, it is checked that not the thing produced in hydrolysis but the Actinomyces concerned itself produces. It sets all over each drawing after drawing 2 . in addition, two sorts of these compounds Serine of a formula (II), N-(2, 3-dihydroxybenzoyl)-, and 3- [2-[(2, 3-dihydroxybenzoyl)amino]-2-propenoate] with 4261-I Formula () Serine of III, N-(2, 3-dihydroxybenzoyl)-, bimol.Ester, and 3-[2-[(2, 3-dihydroxybenzoyl)amino]-2-propenoate] write it as 4261-II, respectively.

[0055] Two sorts of compounds of the aforementioned microorganism origin are in vitro as shown in the following examples of an examination. When it rearranged by the examination system and the prevention ability was evaluated using the mold HCV protease, all are addition concentration 10microM. It sets and enzyme activity is reduced to 50% or less. in addition, since the enzyme activity is not checked to the various proteases which a man produces and exist in the inside of the body, it is caused by prevention of these inside-of-the-body protease -- good -- since better, there is also no colander side effect, and it has the singularity suitable for a medicine use That is, since the prevention ability carried out to the various serine proteases of the man origin is very low, and it has the prevention ability which was specifically excellent to the HCV protease, in addition all are the compounds of low molecular weight comparatively and it has water solubility, the blood drug concentration a curative effect is accepted to be also in the inside of the body can be attained, and it can use as an active principle of a medicine constituent, i.e., an anti-hepatitis C virus agent, and a hepatitis C therapeutic drug.

[0056] In one mode of the hepatitis C therapeutic drug of this invention, as an active principle which shows hepatitis C virus protease inhibitory action, although N-substitution benzoylamino acid derivative shown by the aforementioned general formula (IV), (V), or (VI) or its salt permitted pharmacologically is used, the dosage forms are suitably chosen according to a medication method. It can also consider as the various dosage forms which can dissolve in the carrier permitted pharmacologically, and can also consider as the liquid medicine prescribed for the patient into a blood vessel, such as an injection agent and an intravenous drip agent, or are used for internal use. For example, the dosage forms used for the internal use agent which makes a peptide Mr. compound an active principle in the protease inhibitor of the other virus origins, and similar similar additive composition and form can already be used.

[0057] When a salt with organic bases, such as ammonium salts, such as sodium salt, potassium salt, etc. which used as the corresponding alkali-metal salt the carboxyl group which exists in the compound concerned as the aforementioned salt permitted pharmacologically, for example, or an arginine salt, the basic amino group, a guanidino machine, etc. exist in the compound concerned further, a salt with organic acids, such as a salt with inorganic acids, such as these hydrochlorides, and methanesulfon acid chloride, is mentioned.

[0058] In other modes of this invention hepatitis C therapeutic drug, they are the compound of a formula (II), or a formula. (III) Although compounds or those salts that are permitted pharmacologically are made into an active principle, the dosage forms are suitably chosen according to a medication method. It can also consider as the various dosage forms which can dissolve in the carrier permitted pharmacologically, and can also consider the liquid medicine prescribed for the patient into a blood vessel, such as an injection agent and an intravenous drip agent, or are used for internal use.

[0059] Corresponding to the purpose of medication, and a route of administration, the dosage of the aforementioned compound made to contain in a hepatitis C therapeutic drug takes a patient's age, sex, weight, etc. into consideration, and defines them suitably. For example, when based on the medication in a blood vessel, or internal use, usually a dosage, a medication interval, etc. are defined suitably to exceed at least the concentration which blood drug concentration is made to need for reducing [compound / concerned] the enzyme activity of a HCV protease by half. Generally, it sets to an adult man and is the dosage of per day and the aforementioned compound. 1.0 - 500 mg/kg A single time or multiple times is usually divided and medicated with this total dosage that what is necessary is just to choose from the range. In addition, in blood, since it sets in the process of medicine metabolism, and these aromatic compounds are incorporated by liver and condensed and accumulated out of blood, even if it is low concentration comparatively, the efficiency-concentration in the liver organization of the affected part is maintainable to the high level.

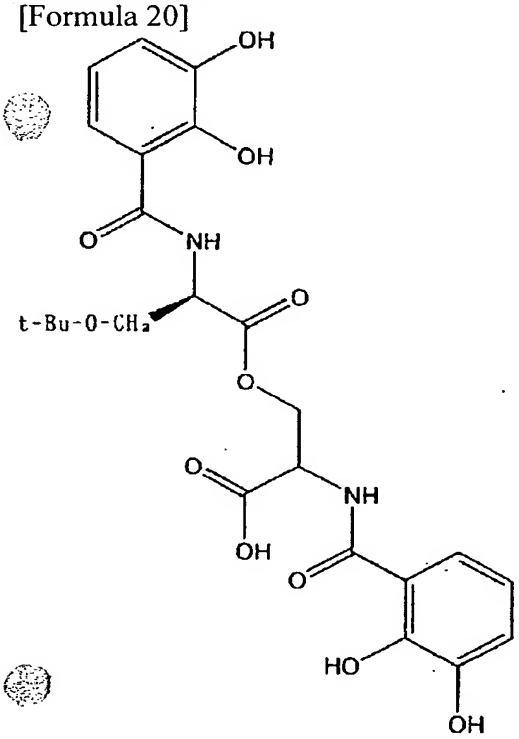
[0060] The new compound of this invention acts as a dipeptide Mr. compound (N-substitution benzoylamino acid derivative) or a tripeptide Mr. compound (N-substitution benzoyl dipeptide derivatives), and shift into the liver cell of the affected part is possible for it by the peptide transport system. In addition, even if it has a peptide chain for a long time in the end of C, it is used as a product of this enzyme digestion in response to digestion by the peptidase as an above-mentioned dipeptide Mr. compound (N-substitution benzoylamino acid derivative) or an above-mentioned tripeptide Mr. compound (N-substitution benzoyl dipeptide derivatives), respectively.

[0061]
[Example] An example is given to below and this invention is explained to it in detail. In addition, the amino acid residue and substitution benzoyl from which the compound described in the following examples 1-12 constitutes it, respectively originate in the amino acid and substitution benzoic-acid derivative which are used as a raw material, and can check the structure with reference to spectrums, such as NMR of these raw material compound, and IR. Moreover, in addition to the peak equivalent to the compound molecule concerned, also in mass analysis, the peak of the fragmentation originating in these substructures is also observed. It checked that it was a compound [purpose] based on these two sorts of results.
[0062] (Example 1) The synthetic method is explained below about a typical thing among the compounds shown by the general formula (IV), (V), and (VI).
N Synthetic [protective-group introduction to hydroxyl of 2 and 3-dihydroxy benzaldehyde] 2 of a -(2, 3-dihydroxybenzoyl) L-serine, 3-dihydroxy benzaldehyde 6.90 g (50 mmol), benzyl chloride 13.8ml (120 mmol), K2CO3 8.29 g (60 mmol), and dehydrated ethanol 65 ml It taught and was made to return for 10 hours. Reaction mixture was cooled and condensed. It melted to ethyl-acetate 125 ml, and washed by water 125 ml and saturation brine 125 ml. With sulfuric-anhydride magnesium, after dehydration, it condensed and dibenzyl-ized derivative [of 15.12g 2 and 3-dihydroxy benzaldehyde], 2, and 3-dibenzyloxy benzaldehyde was obtained as a yellow solid-state.
[0063] [Conversion to the carboxylic acid of the aldehyde by oxidization] 2, 3-dibenzyloxy benzaldehyde They are acetone 60 ml, water 60 ml, and an amidosulfuric acid to 14.33 g (45 mmol). 6.12 g (63 mmol) To the added place, it is a sodium chlorite. 5.41 g (47.3 mmol) It added little by little. After [1 / about] addition Time Heating was continued. After cooling reaction mixture and condensing the acetone, it filtered and the yellow solid-state was obtained. this -- dehydrated ethanol 50 ml it is -- it rinsed, and it was made to dry and 12.63g 2, 2 which is the dibenzyl-ized derivative of 3-dihydroxy benzoic acid, and 3-dibenzyloxy benzoic acid were obtained as a yellow solid-state
[0064] [Manufacture of an acid chloride] 2, 3-dibenzyloxy benzoic acid 3.34 g (10 mmol) Toluene 20 ml and DMF3 It is an oxalyl chloride to the place which added the drop and was ice-cooled. 3.5 ml (40 mmol) It was dropped little by little. It is 1 at 30 minutes and a room temperature under ice-cooling. The time reaction was carried out. The solvent was distilled off and yellow oil was obtained. It was made to crystallize from hexane-ethyl acetate and 2 of 2.98 g, 2 which is the dibenzyl-ized derivative of 3-dihydroxybenzoyl chloride, and 3-dibenzyloxy benzoyl chloride were obtained as a faintly yellow crystal.
[0065] [N-benzoylation reaction] O-benzyl L-serine: It is 2 and 3-dibenzyloxy benzoyl chloride to the place which added Ser(Bzl)-OH 0.10 g (0.51 mmol), NaOH 0.04 g (1.02 mmol), and water 1 ml, and was ice-cooled. It is THF of 0.3 ml about 0.20g (0.56 mmol). What was melted was dropped. It is 1 under ice-cooling. It is 2 at time and a room temperature. The time reaction was carried out. Dilute hydrochloric acid is added slowly and it is pH 2 It adjusts and is 10 ml. Ethyl acetate extracted 3 times. With sulfuric-anhydride magnesium, after dehydration, it condensed and the faintly yellow oil of 0.25 g and the N-(2, 3-dibenzyloxy benzoyl)-O-benzyl serine were obtained.
[0066] It is the compound which was obtained here and by which benzyl protection was carried out Ethanol-ethyl-acetate =20/1 The deprotection was hydrocracked and carried out by Pd/C in inside, and N-(2, 3-dihydroxybenzoyl) serine of a mark was obtained. In addition, as a serine of a raw material, since L-object was used in this example, it was that to which a product also holds the configuration, i.e., an N-(2, 3-dihydroxybenzoyl) L-serine.
[0067] (Examples 2-11) According to the synthesis method of the aforementioned example 1, a series of N-(substitution benzoyl) alpha-amino acid shown in the following table 2 was compounded. In addition, after the compound of a thing and examples 2-7 with which the hydroxyl of phenol nature exists on the substitution benzoyl concerned protected the corresponding substitution benzaldehyde at O-benzyl-ized reaction as above-mentioned, it was guided to target substitution benzoyl chloride. On the other hand, a hydroxyl was not saved on the substitution benzoyl, but with the compound of what exists in a change as a methoxy machine, and examples 8-11, it was prepared to the acid chloride, having used the corresponding substitution benzoic acid of a carboxylic acid as the raw material. The molecular weight shown by mass analysis was as being shown in Table 2.
[0068]
[Table 2]

実施例	化　合　物	分子量
2	N-(2,5-ジヒドロキシベンゾイル) L -セリン	271.2
3	N-(3,4-ジヒドロキシベンゾイル) L -セリン	271.2
4	N-(2,3-ジヒドロキシベンゾイル) L -アラニン	225.2
5	N-(2,3-ジヒドロキシベンゾイル) L -フェニルアラニン	301.3
6	N-(2-ヒドロキシ-3-メトキシベンゾイル) L -セリン	255.2
7	N-(2-ヒドロキシ-5-メトキシベンゾイル) L -セリン	255.2
8	N-(2,3-ジメトキシベンゾイル) L -セリン	269.3
9	N-(2,5-ジメトキシベンゾイル) L -セリン	269.3
10	N-(2,3,4-トリメトキシベンゾイル) L -セリン	299.3
11	N-(3,4,5-トリメトキシベンゾイル) L -セリン	299.3

[0069] (Example 12) The compound of the formula (II) of an N-2 and 3-dihydroxybenzoyl-O-(3-tert-butyloxy-2-(2, 3-dihydroxybenzoylamino) propanoyl) serine above-mentioned [synthetic], and the extremely similar following formula (VIII) : [0070]

[Formula 20]



(VIII)

[0071] It came out and the compound of a mark shown was compounded.

[0072] [Manufacture of O-(2-amino-3-tert-butyloxy propanoyl) serine skeleton] Z-Ser-OBzl 0.329 g (1 mmol), Z-Ser(t-Bu)-OH 0.443 g (1.5 mmol), They are a 1-ethyl-3-(3-N and N-dimethylamino propyl) carbodiimide and a hydrochloride to the place which added HOBt 0.230 g (1.7 mmol) and THF 5ml, and was ice-cooled. (EDC, HCl) 0.326 g was added. It is 1 under ice-cooling. It is 2 at time and a room temperature. Time churning was carried out. It is N-methyl morpholine there. 172microl (1.5 mmol) was added and it agitated at the room temperature for about 20 hours. Ethyl-acetate 20 ml Acetic-acid 0.02 ml It is 0.5 at a room temperature moreover. Time churning was carried out. It is citric-acid solution 20 ml of 1M to this reaction mixture. It is 20 ml after adding. It is 3 with ethyl acetate. Time extraction was carried out. It is NaHCO3 saturated-water solution 20 ml about an organic phase. Saturation brine 20 ml It washed. With sulfuric-anhydride magnesium, after dehydration, it condensed and yellow oil 0.92 g was obtained. The column chromatography (dichloromethane-methanol = 100:1) refined this, and the yellow oil of 0.92 g was obtained.

[0073] It is 0.60 g of these Ethanol-ethyl-acetate =20/1 It hydrocracked by Pd/C in inside, the deprotection of the benzyloxycarbonyl machine of a protective group was carried out, and the resultant of 0.17 g and O-(3-tert-butyloxy-2-amino propanoyl) serine benzyl ether were obtained.

[0074] [N-benzoylation reaction] Obtained resultant It is 2 and 3-dibenzyloxy benzoyl chloride to the place which added NaOH 0.08 g (2.0 mmol) and water 2 ml, and was ice-cooled. It is THF of 0.6 ml about 0.78 g

(2.2 mmol). What was melted was dropped. It is 1 under ice-cooling. It is 2 at time and a room temperature. The time reaction was carried out. Dilute hydrochloric acid is added slowly and it is pH 2 It adjusts and is 20 ml. It is 3 with ethyl acetate. Time extraction was carried out. With sulfuric-anhydride magnesium, after dehydration, it condensed and the yellow oil of 0.70 g was obtained. The column chromatography (dichloromethane) refined and the oil of the thin yellow of 0.41 g was obtained. It is the obtained benzyl protection compound Ethanol-ethyl-acetate =20/1 The deprotection was hydrocracked and carried out by Pd/C in inside, and the compound of a mark was obtained. in addition, the result of mass analysis -- molecular weight -- 520.5 it is -- things were checked

[0075] Formula [which is used for the HCV protease inhibitor of this invention / aforementioned] (II) (Example 13) Reach. (III) It is shown in the following examples of an examination that two sorts of compounds can all attain sufficient prevention in low concentration. In addition, natural HCV protease Cpro-2 The polypeptide aforementioned within a host cell Since it is difficult to be produced as p70 and to receive in large quantities, it is this HCV protease. Cpro-2 By the genetic engineering-technique, the rearranged type hepatitis C virus protease which has equivalent protease activity was produced using the transformation microorganism, and it was used. The synthetic peptide which similarly has the specific amino acid sequence which a natural HCV protease cuts as a protein matrix (peptide) was used.

[0076] The example of examination Homoto estimated the prevention ability to enzyme activity by making into a substrate the synthetic peptide which has the specific amino acid sequence which a natural HCV protease cuts, or a recombination protein matrix, using a rearranged type HCV protease as a HCV protease.

[0077] A rearranged type HCV protease builds the annular plasmid vector which comes to contain DNA which carries out the cord of this rearranged type HCV protease according to the following procedures, produces the transformation microorganism which has the productivity of this rearranged type HCV protease that introduces and comes to carry out the transformation of the annular plasmid vector, cultivates this transformation microorganism, from the culture, isolates and extracts this rearranged type HCV protease, and prepares it (see JP,9-9961,A etc.). in addition, the cord of the protein of the HCV origin, such as a HCV protease, is carried out cDNA From the viral genome (RNA of + chain) of HCV, reverse transcription is carried out and it is extracted. the amino-acid-residue array which corresponds [which corresponds and nucleic-acid-arranges] it is already reported to reference (A.Takamizawa et al., J.Virol., 65, and 1105-1113 (1991) --) Refer to N.Kato et al., Proc.Natl.Acad.Sci.USA, 87, 9524-9528, etc. (1990). This invention persons referred to the HCV-J type genome nucleic-acid array (see N.Kato et al., Proc.Natl.Acad.Sci.USA, 87, 9524-9528, etc. (1990)).

[0078] [Construction of the annular plasmid vector which comes to contain DNA which carries out the code of the rearranged type HCV protease and manufacture of the transformation microorganism which introduced this plasmid vector] It is a HCV protease first. Cpro-2 The preliminary research which aims at the specification of an amino-acid-sequence portion which demonstrates protease activity is made, and some acquired knowledge is reported. On the amino acid sequence written from the N end of the polypropylene theine translated from the genome of a HCV viral genome, On the end field of N of NS3 (P70) portion of a non-structural gene field, and a concrete target Ala1027 - Ser1207 portion (see J.Viorogy, 68, and 8147-8157 (1994)), or -- Gly1049 - Thr1215 portion (see Gene, 145, 215-219 (1994), and JP,6-315377,A) It is reported that it accepts and comes out and there is protease activity. It is based on these knowledge and is NS3 portion of a non-structural gene field, P70 [i.e.,]. The cDNA fragment which carries out the code of the field called was extracted. this p70 ; 5' of the DNA fragment which carries out the code of Ala1027-Thr1658 it transposes to a side in the linker fragment 1 shown in drawing 3 (above) -- just before Ala1027 -- start codon while adding ATG (Met) -- restriction enzyme EcoR I The cleavage site was introduced. simultaneously, it replaces in the linker fragment 2 which shows 3' side to drawing 3 (below) -- while introducing a stop codon TAA immediately after Thr1658 -- 3' the non-translating region of a side lower stream of a river -- restriction enzyme Hind III The cleavage site was also introduced. The obtained DNA fragment is a restriction enzyme to an end. EcoR I Hind III The amputation stump was formed, respectively.

[0079] The fusion protein which connected the HCV protease in the end of C of the maltose joint protein (MBP;maltose-binding protein) of the Escherichia coli origin was used for the recombinant HCV protease. The MBP manifestation plasmid vector marketed () [NEW] ENGLAND BIOLABS (NEB) -- cutting Make pMAL-c2 Restriction enzyme (C. -d.Guan etal., Gene, 67, and 21-30 (1988) --) EcoR I Hind III With reference to C.V.Maina et al., Gene, 74, 365-373 (1988), etc., the vector fragment containing DNA (mal E) which carries out the code of the MBP obtained is refined. This vector fragment and above p70 ; It is the DNA fragment which carries out the code of Ala1027-Thr1658 T4 It connects by the DNA ligase and is a plasmid vector. pMAL-CP is obtained. The plasmid vector obtained is the above. DNA which carries out the code of the MBP-p70 fusion protein It originates in pMAL-c2. It has on a tac promotor's (Ptac) lower stream of a river, and has an ampicillin resistance gene (Ampr) as a marker gene. this -- MBP-p70 fusion-protein expression vector

pMAL-CP -- using -- Escherichia coli A transformation is introduced and carried out to HB [101 stocks of]. Screening by the western blotting using the ampicillin resistance by the marker gene and a rabbit anti-MBP antibody is performed, and it is this expression vector. The recombination bacillus which has pMAL-CP was obtained.

[0080] [Manufacture of a MBP-p70 fusion-protein type rearranged type HCV protease] Rearranged type HCV [activity / from cultivation production and the culture of the fusion protein by the recombination bacillus] The following procedures perform separation and refining of a protease. 2xYT of 500 ml which added the ampicillin for the recombination bacillus is used for a culture medium, and shaking culture is carried out in 5L ********** 30 degrees C and overnight (about 14 hours). It is MBP-p70 to during this period and a logarithmic growth phase. In order to guide the manifestation of a fusion protein, it is an isopropyl to a culture medium. - Beta-D - It is set to final concentration 1 mM, and amount addition of the thio galactopyranoside (IPTG) is carried out. Then, 30 degrees C and 14-hour shaking culture are performed, centrifugal separation of the culture medium obtained is carried out, and it carries out a harvest. A biomass is crushed according to an ultrasonic wave after a harvest, after that, by centrifugal separation, an insoluble fraction is separated and the supernatant liquid containing fusibility protein is obtained.

[0081] The 137.5ml of the aforementioned supernatant liquids extracted from culture medium 2L MBP-p70 which is adsorbing on a column after it applies to the amylose column (product made from 32x63mm; amylose range NEW ENGLAND BIOLABS) which equilibrated with buffer liquid (20 mM Tris-HCl, pH 7.4, 0.2M NaCl, 1 mM EDTA) and this buffer liquid washes The fusion protein was eluted with this buffer liquid which added 10mM maltose. This adsorption fraction is condensed with an ultrafiltration (YM30 membrane film). About this concentration liquid, they are 0.2 M NaCl and 0.1 M. Sephacryl equilibrated by sodium phosphate and pH 7.2 buffer liquid S-300 HR A load is carried out to a column (product made from Pharmacia), and a gel filtration chromatography is performed. The 400ml of the rates of flow was set to /h, and it set fractionation each to 20ml. Target MBP-p70 The fraction containing a fusion protein is extracted.

[0082] MBP-p70 which gave this gel filtration The fraction containing a fusion protein is again condensed by the ultrafiltration membrane. About this, it is 20 mM. Sephadex equilibrated by the sodium phosphate buffer solution and pH 7.2 (buffer liquid B is called henceforth) G-25 A load is carried out to a column and it is buffer liquid B about a solvent. MBP-p [finishing / refining / exchangeably]70 A fusion protein solution is obtained. Finally, it is final concentration about a MBP-p70 fusion-protein solution [finishing / refining]. After adding a glycerol so that it may become 50 %, and adjusting protein concentration to 1mg / ml, it saves in -80 **.

[0083] The [manufacture method of a recombination protein matrix] Since the protein matrix which a natural HCV protease cuts is the gene product of a HCV virus, it is changed to this protein matrix and a recombination protein matrix including the amino acid sequence which a HCV protease cuts is used for it. The partial array (cutting array; Cleavage Sequence CS) shown in drawing 4 corresponding to this HCV protease cutting array (NS5A/NS5B) is specifically inserted in between in the N end of the C end of MBP, and the adenylate kinase (it is henceforth called ADK for short.), this fusion protein (it is henceforth called for short MBP-NS5A/5 B-ADK.) of MBP and ADK is rearranged, and it uses as a protein matrix.

[0084] ** The recombination Escherichia coli used for the manifestation of MBP-NS5A / 5 B-ADK fusion protein was obtained by the following method. The MBP manifestation plasmid vector marketed () [NEW] ENGLAND BIOLABS (NEB) -- cutting Make pMAL-c2 Restriction enzyme (C.-d.Guan et al., Gene, 67, and 21-30 (1988) --) Xmn I Hind III With reference to C.V.Maina et al., Gene, 74, 365-373 (1988), etc., the vector fragment containing DNA (mal E) which carries out the code of the MBP obtained is refined. ADK expression vector containing this ADK gene (see T.Hibino et al., J.Biotechnol., 32, 139-148, etc.) by which the clone was carried out on the other hand in the DNA fragment which carries out the code of the ADK pMKAK3 etc. (see a publication-number 1 No. -51087 official report, the publication-number 1 No. -51088 official report, etc.) etc. -- restriction enzyme EcoR I Hind III It cuts and refines. the synthetic linker which shows the DNA fragment which carries out the code of the ADK to the vector fragment containing DNA (mal E) which carries out the code of the above MBP to drawing 4 is minded -- T4 It connects by the DNA ligase. The plasmid vector obtained is the above. DNA which carries out the code of MBP-NS5A / the 5 B-ADK fusion protein It originates in pMAL-c2. It has on a tac promotor's (Ptac) lower stream of a river, and has an ampicillin resistance gene (Ampr) as a marker gene. this -- MBP-NS5A / 5 B-ADK fusion protein expression vector pMAL-AK2 using -- Escherichia coli A transformation is introduced and carried out to HB [101 stocks of]. Screening by the western blotting using the ampicillin resistance by the marker gene and a rabbit anti-MBP antibody is performed, and it is **. MBP-NS5A / 5 B-ADK fusion protein expression vector pMAL-AK2 The recombination bacillus which it has was obtained. In addition, the code of the amino-acid-sequence portion used as a substrate is carried out by this synthetic linker portion.

[0085] This vector pMAL-AK2 About the recombination bacillus which it has, it is 500 ml. The culture

medium containing 2xYT is used and it is 5 L **********. 30 ** and a night (abbreviation 14 time) Shaking culture is carried out. It is final concentration to the meantime and a logarithmic growth phase. 1 mM Becoming amount IPTG It adds and the manifestation of this MBP-CS-ADK fusion protein is guided. Then, from a culture, a harvest is carried out, it ****, the ultracentrifuge of the biomass is carried out, and it is sonic sup 44 ml. It obtained. About this fusibility fraction, it is 20 mM. 10 mM after it puts on amylomaize-SUKARAMU which equilibrated with the tris hydrochloric-acid buffer (pH 7.4, 0.2 M NaCl, and 1 mM EDTA) and this buffer washes An adsorption fraction is eluted with this buffer containing a maltose. It is this adsorption fraction Sephadex G-25 It uses and is 20 mM. To a tris hydrochloric-acid buffer (pH 7.6, 60 nM NaCl), after carrying out solvent substitution, it condenses with an ultrafiltration (YM30 membrane). condensed ** Glycerol of volume [solution / of MBP-NS5A / 5 B-ADK fusion protein] 1/500 volume 1 M DTT -- in addition, it saves at -20 degree C In addition, this recombination substrate liquid is protein concentration. 2.81 mg/ml It adjusted. In addition, aforementioned MBP-p70 Fusion protein expression vector pMAL-CP and ** MBP-NS5A / 5 B-ADK fusion protein expression vector pMAL-AK2 The SDS-PAGE analysis result of a ** type view and both fusion proteins is shown in drawing 5 as reference.

[0086] [Synthetic substrate] The synthetic substrate which is the peptide which has an amino acid sequence corresponding to a HCV protease cutting array (NS5A/NS5B) and which carries out the following was created.
 composition substrate (S3) Abz-Glu-Asp-Val-Val-Glu-Cys-Ser-Met-Ser-Tyr-NH2 (Abz: 2-amino benzoyl) -- this synthetic substrate It is Cys-Ser when cut by HCV protease. Two sorts of peptide fragments which were cut in between and which carry out the following are obtained.

Peptide fragment Abz-Glu-Asp-Val-Val-Glu-Cys-OH Ser-Met-Ser-Tyr-NH2 [0087] [HCV protease activity assay of in vitro using the recombination protein matrix] HCV protease fusion protein MBP-p70 prepared by the aforementioned method And recombination protein matrix The HCV protease activity assay of in vitro was performed using MBP-NS5A/5 B-ADK. An example of an assay procedure is described below.

[0088] What diluted above-mentioned preservation enzyme liquid, the protein concentration of 1mg / thing of ml with the buffer solution for dilution (10 mM NaH2PO4, 50 % glycerol, and pH7.2) 5 times is used for an enzyme solution. It is once DMF about the specified quantity of an examined substance. It dissolved and considered as sample liquid. This sample liquid 1 mul TSC Buffer-solution (50 mM Tris-HCl, 30 mM NaCl, 5 mM CaCl2, and pH8.5) 34microl In addition, stirring mixture is carried out. Subsequently, MBP-NS5A / 5 B-ADK recombination substrate (protein concentration 2.81 mg/ml) 10 mul and enzyme solution 5microl It adds one by one and a reaction is performed at 37 degrees C for 1 hour. Then, sample solution for SDS-PAGE to reaction mixture 50 mul In addition, a reaction is stopped.

[0089] To this liquid, it is 2-mercaptoethanol. 3.3microl It adds. This mixed liquor to 10microl It extracts, and the argentation is SDS- PAGE(ed) and carried out. It generates by this recombination substrate and its protease digestion. The intensity of the band which ADK gives is measured with a densitometer. Namely, recombination substrate With the amount of survival of MBP-NS5A/5 B-ADK, it newly generates. Enzyme activity is calculated based on each band strength measured in the amount of ADK.

[0090] On the other hand, it changes to the sample liquid of an examined substance, and a ratio with the enzyme activity at the time of adding the sample liquid of an examined substance which carries out the remainder is calculated by making into a reference group what carried out the same operation using DMF. Incidentally, protease prevention ability of each examined substance is taken as 100% of rates of prevention, when the enzyme activity which carries out the remainder 0% of rates of prevention when the enzyme activity which carries out the remainder is the same as a reference group is zero.

[0091] [HCV protease activity assay in in vitro using the synthetic substrate] What diluted above-mentioned preservation enzyme liquid, the protein concentration of 1mg / thing of ml with the buffer solution for dilution (10 mM NaH2PO4, 50 % glycerol, and pH7.2) 40 times is used for an enzyme solution. It is once DMF about the specified quantity of an examined substance. It dissolved and considered as sample liquid. This sample liquid 2microl TSCG buffer-solution (50 mM Tris-HCl, 30 mM NaCl, 5 mM CaCl2, 50 % glycerol, and pH8.5) 10microl and TSC buffer-solution (50 mM Tris-HCl, 30 mM NaCl, 5 mM CaCl2, and pH 8.5) 58microl In addition, stirring mixture is carried out. Subsequently, enzyme solution 10microl 125 after adding and performing pre incubation processing for 10 minutes at a room temperature muM It is 20microl about a synthetic substrate solution. It adds and a reaction is performed at 37 degrees C for 1 hour. To reaction mixture, it is 0.5 % TFA 100. mul In addition, a reaction is stopped. It is an antiphase, respectively about the fragment cut by the enzyme reaction of the synthetic substrate which remains after a reaction halt and into the reaction mixture, and a synthetic substrate. HPLC It analyzes. The conditions of Antiphase HPLC are an antiphase C4. Using a column (4.6 mm bore x 5.0 cm and VyDac), elution is 14% acetonitrile addition. It considered as the conditions of TFA (for 2 minutes) 0.1%. In addition, sample liquid 2microl of an examined substance It changes and is DMF2microl. It used, the same operation as the above was performed, and the rate of prevention

was calculated by making this into a reference group.
[0092] Final concentration 10microM of the inside of reaction mixture, and a test compound The prevention activity of each examined substance when carrying out is shown in Table 3. In addition, evaluation used the method of using a recombination substrate. A formula (II), formula (III) In addition to a compound, the result of 2, 3-dihydroxy benzoic-acid, 1, and 2-dihydroxybenzene (catechol) is also shown collectively.
[0093]
[Table 3]

被 験 物 質	阻害活性（%）
式（II）の化合物	79.5
式（III）の化合物	84.2
2,3-ジヒドロキシ安息香酸	11.5
カテコール	86.8

[0094] They are these formulas (II) or a formula as a result is shown in Table 3. (III) It turns out that two sorts of shown compounds are both the inhibitors which were excellent to the HCV protease. Specifically, the rate of prevention has reached to about 80% in the aforementioned result. on the other hand, it turns out that 2 [characteristic of the substructure of a formula (I)] and the 3-dihydroxy benzoic acid itself are what is markedly alike and is inferior although prevention activity is seen It can be guessed that it forms the amino acid residue and hydrogen bond of an active center of a HCV protease using the oxygen atom of the hydroxyl which exists as a substituent on the benzene ring since 1 and 2-dihydroxybenzene (catechol) shows the rate of prevention of the same grade as these compounds. That is, it is thought using the substructure of a formula (I) that it configurates in the active center of a HCV protease.
[0095] two sorts of these inhibitors are the purposes which consider according to what mechanism the enzyme activity of a HCV protease is checked, and boil various addition concentration of an inhibitor, it changes, and the dependency over a synthetic substrate concent is measured, from the result [Lineweaver-Burk plot / the dependency over the addition concentration and a synthetic substrate concent / result], prevention is judged to be competitive inhibition and it is presumed to a HCV protease that a reaction with a substrate is checked by configurating at the enzyme-activity point Moreover, when the concentration which checks the enzyme activity of a HCV protease to 50% was calculated based on the result shown in drawing 6 in the system which used the synthetic substrate, IC50 value of the compound of a formula (II) is about 6.5. muM, formula (III) IC50 value of a compound is about 4.0. It was able to be found with muM, respectively.
[0096] In addition, although the aforementioned compound showed the prevention activity which was extremely excellent to the HCV protease, it only verified variously that peculiar to the other man it was that prevention activity is shown slightly to the serine protease. Specifically, a trypsin, a chymotrypsin, an elastase, a plasmin, a thrombin, a kallikrein, and the enzyme activity prevention ability to seven sorts of Factor Xa were measured. In addition, the cow serum albumin is added by the system of reaction by the evaluation system of a thrombin and Factor Xa. In addition, same evaluation was performed also about the serine protease of the herpes-simplex-virus 1 type (HSV-1) origin. as an example of an evaluation result being shown in drawing 7 and drawing 8 -- each inhibitor -- the inside of reaction mixture -- final concentration 100microM Although there is an inclination for enzyme activity to be suppressed slightly when it adds, as compared with the prevention ability to a HCV protease, it is markedly alike, and it is judged that it is low. That is, it turns out that it is what checks enzyme activity specifically to a HCV protease. As reference, it is final concentration 10microM in reaction mixture. The rate of prevention at the time of adding and the addition concentration of each enzyme are collectively shown in Table 4.
[0097]
[Table 4]

セリンプロテアーゼ		阻害率 (%)	
種類	酵素濃度	式 (II) の化合物	式 (III)の化合物
トリプシン	0.2U/ml	4	5
キモトリプシン	0.004U/m	4	5
エラスターゼ	4U/ml	10	10
トロンビン	0.05U/ml	< 1	< 1
カリクレイン	0.1U/ml	7	11
ファクターXa	0.1U/ml	< 1	< 1
プラスミン	0.4U/ml	< 1	< 1

[0098] More specifically a HCV protease Are inherent in the substructure of an enzyme activity point as an indispensable portion in the array of His1083-X22-Val1104-X-X-Asp1107-X55-Gly1163-X-Ser1165-Gly1166-X-Pro1168-X9-Gly1178. His1083, Asp1107, and Ser1165 which exist in this amino acid sequence are enzyme activity point;His-Asp-Ser of a serine protease. It is thought that the substructure is formed. Probably at the time of peptide linkage cutting of a substrate, it can be presumed from the aforementioned side-chain imidazole ring of His1083 that a proton is supplied. Some prevention ability is shown as a trypsin, a chymotrypsin, an elastase, a plasmin, a thrombin, a kallikrein, and seven sorts of proteases of Factor Xa are also shown in the aforementioned table 3, since the active spot similar to the substructure of His-Asp-Ser focusing on enzyme activity is formed. However, since the configurations of the amino-acid-residue side chain of the circumference differ so that it may be predicted from the singularity of a cutting array, it is judged that prevention activity has brought a greatly different result. However, by the various intervals between roots of HCV, the structure near [this] the enzyme activity point is saved very well, and equal prevention activity will be shown also to the HCV protease originating in HCV(s) other than a HCV-J stock.
[0099] In addition, the aforementioned formula used for the HCV protease inhibitor of this invention (VIII) Each verified that sufficient prevention could be attained in low concentration according to the above-mentioned examining method also about the compound shown by the shown compound and the general formula (IV), (V), and (VI). In addition, the protease prevention ability was evaluated about the flavonoid which has L-serine, D-serine, various dihydroxy benzoic-acids, trihydroxy benzoic-acids, L-serine, D-serine, 3, and 4-dihydroxy phenyl group for reference. To Table 5, it is final concentration 10microM in reaction mixture. In addition to the rate of prevention at the time of adding, about some compounds in which high prevention ability is shown, it is final concentration 1microM. The result at the time of adding is combined for contrast, and is shown.
[0100]
[Table 5]

被験物質 / 添加濃度	阻害活性（%）	
	10μM	1μM
式(VIII)の化合物	84.2	18.4
N-(2,3-ジヒドロキシベンゾイル)セリン	41.1	21.5
N-(2,5-ジヒドロキシベンゾイル)セリン	56.6	43.4
N-(3,4-ジヒドロキシベンゾイル)セリン	36.8	9.0
N-(2,3-ジヒドロキシベンゾイル)アラニン	52.0	17.3
N-(2,3-ジヒドロキシベンゾイル)フェニルアラニン	68.1	37.0
N-(2-ヒドロキシ-3-メトキシベンゾイル)セリン	21.3	7.3
N-(2-ヒドロキシ-5-メトキシベンゾイル)セリン	19.4	—
N-(2,3-ジメトキシベンゾイル)セリン	5.1	—
N-(2,3,4-トリメトキシベンゾイル)セリン	9.1	—
N-(3,4,5-トリメトキシベンゾイル)セリン	11.3	—
L-セリン	<1.0	—
D-セリン	<5.0	—
1,2-ジヒドロキシベンゼン	86.8	—
1,2-シクロヘキサンジオール	<1.0	—
1,3-ジヒドロキシベンゼン	4.1	—
1,3,5-トリヒドロキシベンゼン	22.8	—
1,2,3-トリヒドロキシベンゼン	96.3	—
2,4,6-トリヒドロキシベンゾアルデヒド	39.2	—
-ヒドロキシ 安息香酸	9.5	—
2,3-ジヒドロキシ安息香酸	11.5	—
2,5-ジヒドロキシ安息香酸	70.0	—
2,4-ジヒドロキシ安息香酸	14.0	—
2,6-ジヒドロキシ安息香酸	18.7	—
3,5-ジヒドロキシ安息香酸	20.0	—
3,4-ジヒドロキシ安息香酸	50.1	—
3,4,5-トリヒドロキシ 安息香酸	96.1	—
3,4,5-トリヒドロキシ 安息香酸メチル	92.3	—
(3,4-ジヒドロキシフェニル)-1,2-エタンジオール	96.0	—
3,4-ジヒドロキシフラボン	32.1	—
(+)-カテキン (2R,3S)	42.6	—

[0101] It turns out that the hepatitis-C-virus protease prevention activity which the substitution benzoylamino acid derivatives of this invention show from the aforementioned result is mainly a thing originating in the structure of a substitution benzoyl although the structure of the amino acid residue which constitutes it also involves. That is, in a substitution benzoylamino acid derivative, it is judged that the structure of the carboxyl terminus may not be essential to prevention ability, and it may be what structure in an interaction with an enzyme unless it becomes steric hindrance. Similarly, unless it becomes remarkable steric hindrance also about the side chain of an amino acid residue, it is judged that you may be what structure. However, about the side chain of this amino acid residue, a result by which the hydrocarbon group which is rich hydrophobic is imagined to be more suitable is brought.

[0102]

[Effect of the Invention] The hepatitis C virus protease inhibitor of this invention is boiled in the height of the prevention ability, in addition has water solubility, and since molecular weight is also comparatively low, it is suitable for a medicine constituent. namely, the ring which shows a hydrophobic property -- having -- in addition -- and since it has water solubility, it has the advantage which can make a medicine absorb to the inside of the body also in various medication forms it is low molecular weight especially comparatively -- in addition, since it has the property of a hydrophobic property and a hydrophilic property, also make easy shift into the host cell of the liver with which a virus is infected -- the prevention activity can be demonstrated From these advantages, the hepatitis C therapeutic drug of this invention can demonstrate a high curative effect.

[Translation done.]

* NOTICES *

1.This document has been translated by computer. So the translation may not reflect the original precisely.
2.**** shows the word which can not be translated.
3.In the drawings, any words are not translated.

DESCRIPTION OF DRAWINGS

[Brief Description of the Drawings]
[Drawing 1] Actinomyces Streptomyces longisporous JCM4261 Drawing showing the HPLC analysis result of the acid extraction fraction in a stock culture supernatant.
[Drawing 2] Actinomyces Streptomyces longisporous JCM4261 Drawing showing the HPLC analysis result of a stock culture supernatant.
[Drawing 3] DNA which carries out the code of the recombination HCV protease Drawing showing the base sequence of the linker fragment 1 used for creation of a fragment, and a fragment 2.
[Drawing 4] Drawing showing the base sequence of the linker fragment which carries out the code of the tting array in a recombination substrate.
[Drawing 5] A recombination HCV protease and drawing in which rearranging and showing the expression vector of a substrate typically.
[Drawing 6] The compound of a formula (II), and formula (III) Drawing showing the dependency over the compound addition concentration concerned in the enzyme-inhibition ability to the HCV protease of a compound.
[Drawing 7] Compound of a formula (II), and formula (III) The trypsin of a compound, a chymotrypsin, an elastase, and HSV-1 Drawing which compares the enzyme-inhibition ability to an origin serine protease.
[Drawing 8] The compound of a formula (II), and formula (III) Drawing which compares the enzyme-inhibition ability to the plasmin of a compound, a thrombin, a kallikrein, and Factor Xa.

[Translation done.]

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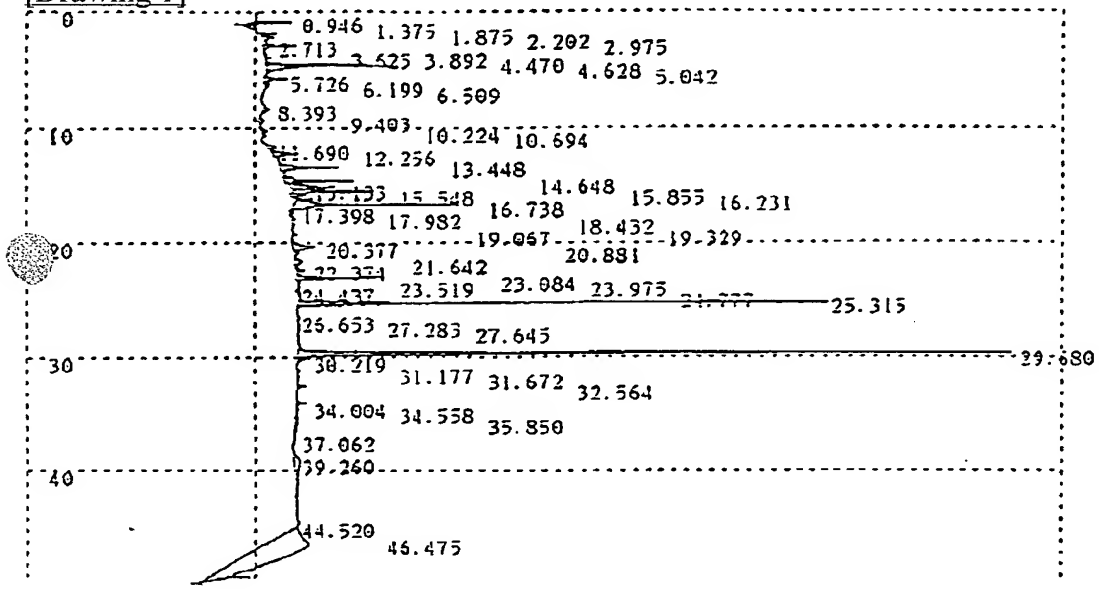
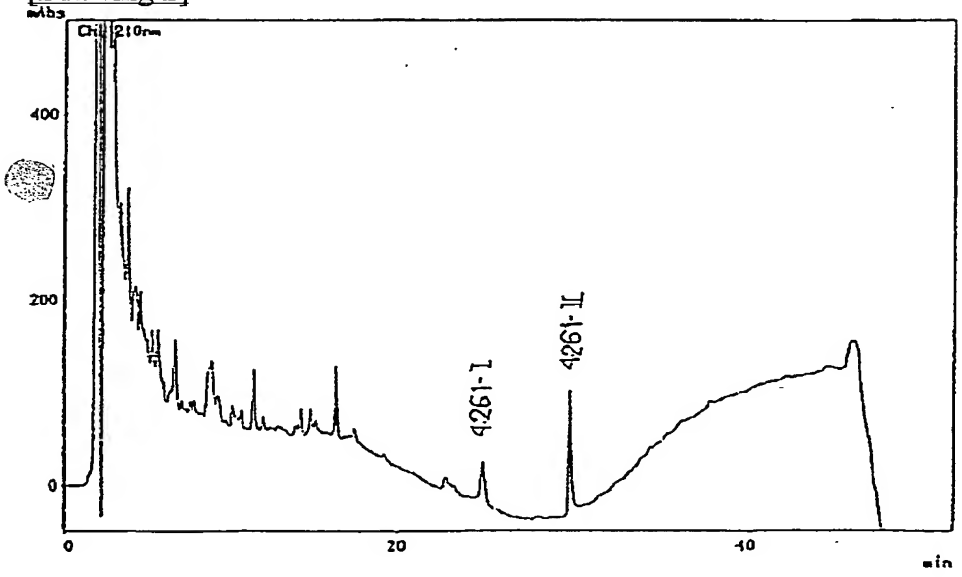
DRAWINGS

[Drawing 1]



[Drawing 2]



[Drawing 3]

N末端合成リンカーの塩基配列

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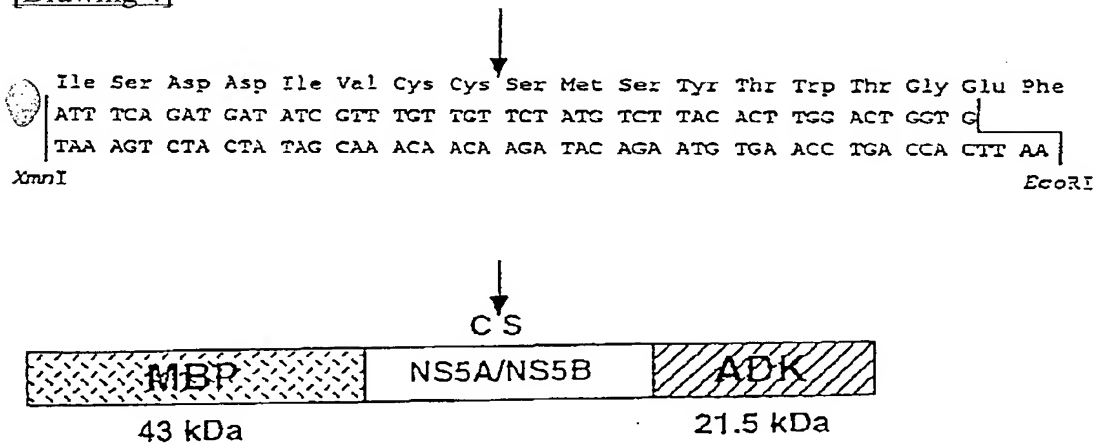
                1027
          Met Ala Pro Ile Thr Ala Tyr Ser Gln Gln Thr Arg Gly Leu
AA TTC ATG GCG CCT ATC ACG GCC TAT TCC CAA CAA ACG CGG GGC CTG CT
   G TAC CGC GGA TAG TGC CGG ATA AGG GTT GTT TGC GCC CCG GA
EcoRI                                                      BglI
```

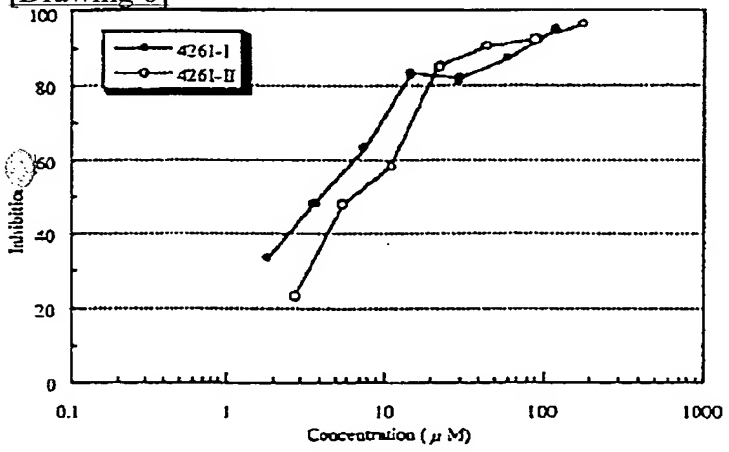
C末端合成リンカーの塩基配列

```
                                                  1658
          Ser Ala Asp Leu Glu Val Val Thr ***
   TG TCG GCC GAC CTG GAG GTC GTC ACT TA
ACG TAC AGC CGG CTG GAC CTC CAG CAG TGA ATT CGA
EcoT22I                                    HindIII
```

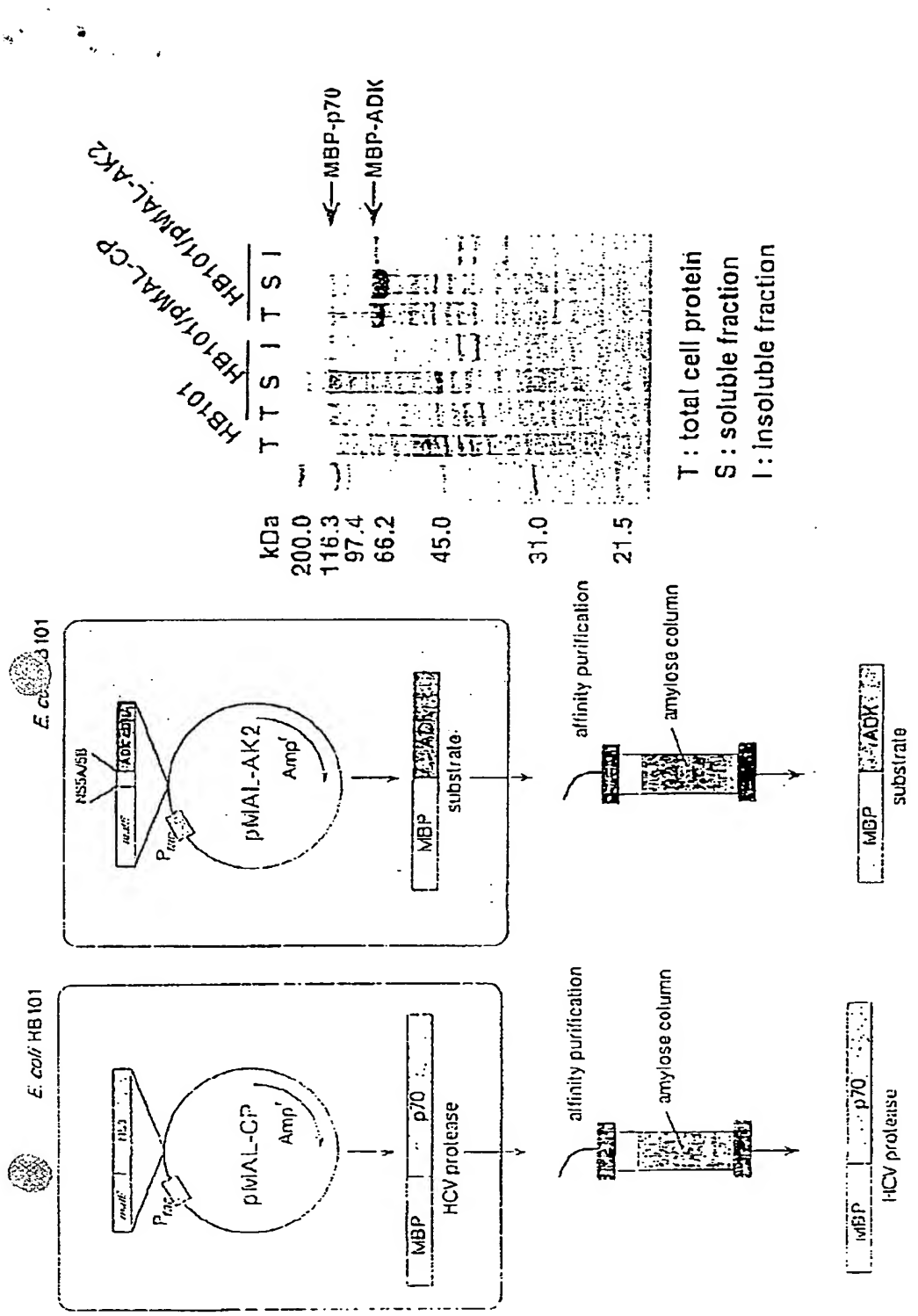
[Drawing 4]



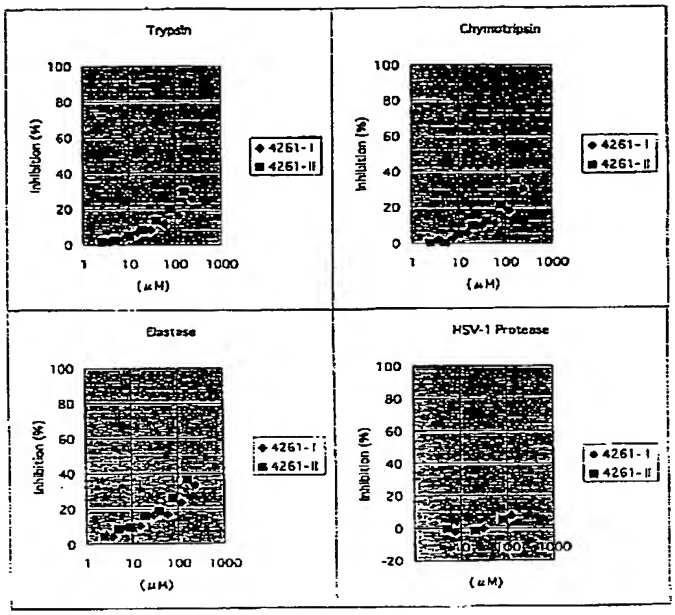
[Drawing 6]



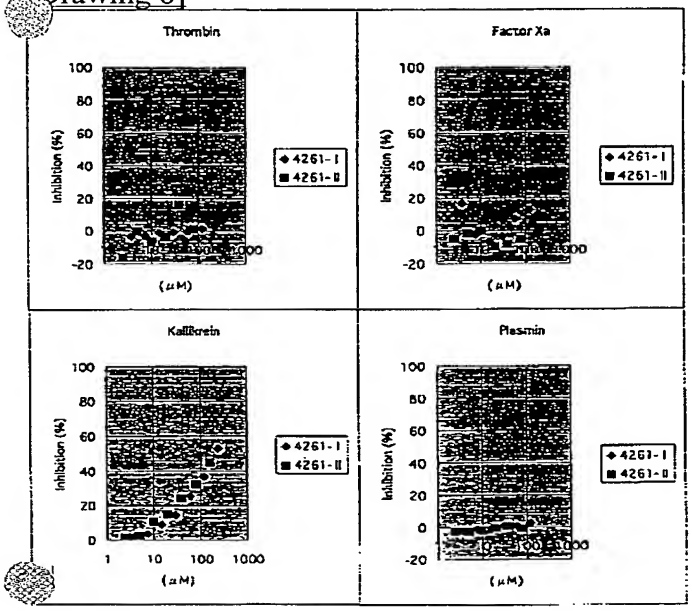
[Drawing 5]


HB101
HB101/pMAL-CP
HB101/pMAL-AK2
T T S I T S I
MBP-p70
MBP-ADK
kDa
200.0
116.3
97.4
66.2
45.0
31.0
21.5
T : total cell protein
S : soluble fraction
I : insoluble fraction
E. coli HB101
pMAL-CP
Amp'
MBP
p70
HCV protease
affinity purification
amylose column
pMAL-AK2
substrate
ADK

[Drawing 7]



[Drawing 8]



[Translation done.]